Indian Journal of Chemistry
Vol. 37B, April 1998, pp.352 - 357

Carbon oxysulphide: A novel reagent for the synthesis of 4-amino/anilino-3-aryl/aryloxymethyl/thiophenoxymethyl-1,2,4-triazolin-5-ones and 5-arylamino-2-mercapto-1,3,4-oxadiazoles

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Received 17 July 1995; accepted (revised) 26 May 1997

Acid hydrazides **1** on reaction with carbon oxysulphide in the presence of alcoholic KOH afford potassium β-acylthiocarbazinates **2** which on further reaction with hydrazine hydrate or phenylhydrazine give 4-amino-3-substituted-1,2,4-triazolin-5-ones **3** and 4-anilino-3-substituted-1,2,4-triazolin-5-ones **5** respectively. 4-Substituted thiosemicarbazides **6** on treatment with carbon oxysulphide in the presence of alc NaOH afford sodium β-(*N*-arylthiocarbamyl)thiocarbazinates **7** which on cyclisation in the presence of ethanolic NaOH afford 5-arylamino-2-mercapto-1,3,4-oxadiazoles **8**. On reaction with benzyl chloride in the presence of NaOH **8** isomerise to 3-benzylmercapto-4-substituted-1,2,4-triazolin-5-ones **9**.

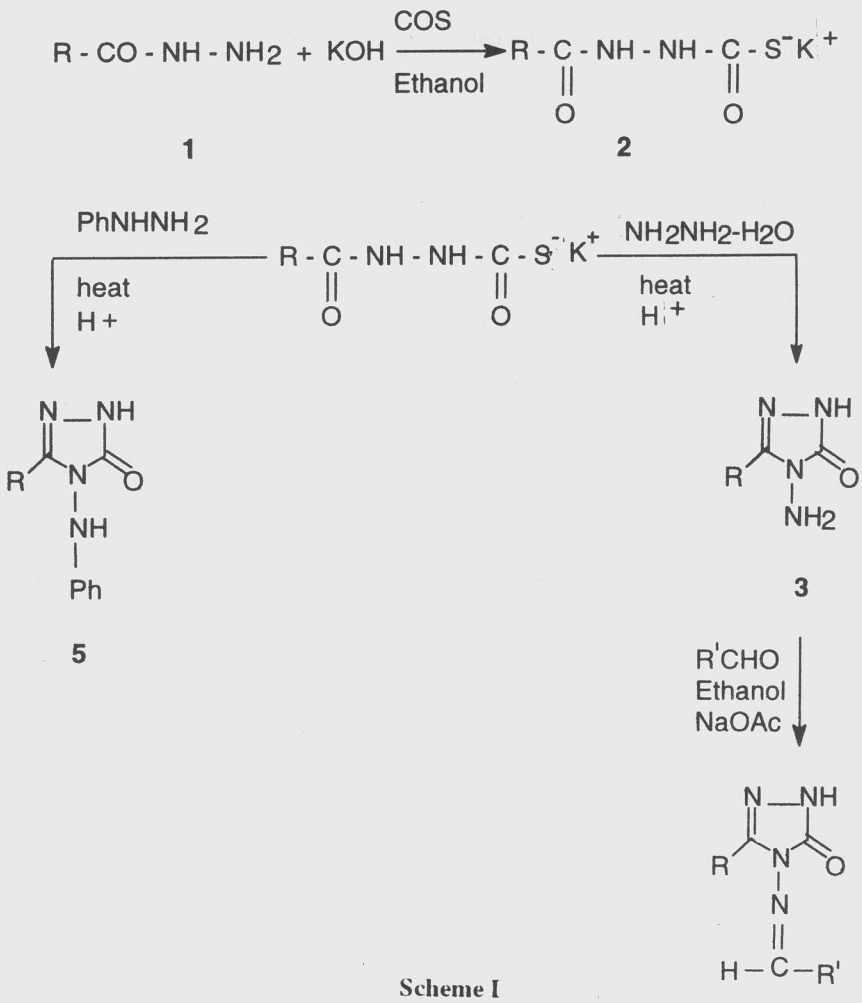
Acid hydrazides and 4-substituted thiosemicarbazides can be converted into derivatives that are useful precursors of 5-membered heteroaromatic systems, particularly pyrazoles, 1,2,4-triazoles and 1,3,4-thiadiazoles[1-5]. Kurzer and Esmail[6] have prepared 4-amino-3-phenyl-1,2,4-triazolin-5-one **3** as a side product in very low yields by cyclisation of thiobenzoyl-1-carbohydrazide. Milcent and Redeuilh[7] prepared **3** by the action of aryliminoethers on carbohydrazide in which other products were also formed. Chande and Karnik[8] have reported the formation of 2-benzylmercapto-1,3,4-oxadiazoles in a single step reaction from 4-substituted thiosemicarbazide **6** and carbon oxysulphide. 1,3,4-oxadiazoles were then isomerised to the corresponding 4-substituted-3-benzylmercapto-1,2,4-triazolin-5-one **9**.

In continuation of our work on carbon oxysulphide[8,9], we now report a simple and cost effective synthesis of 4-amino-3-aryl/aryloxymethyl/thiophenoxymethyl-1, 2, 4-triazolin-5-ones **3**, 4-anilino-3-aryl/aryloxymethyl/1,2,4-triazolin-5-ones **5** and 5-substituted-2-mercapto-1,3,4-oxadiazoles **8**. The structural assignments of the products were based on elemental analyses (C,H and N, Table I) and IR, PMR and ^{13}C-NMR data. The spectral data of only representative compounds are given in the Experimental Section.

Acid hydrazides **1** in alc KOH were treated with a dry stream of carbon oxysulphide when potassium β-acyl-thiocarbazinates **2** were obtained which were then heated with hydrazine hydrate (99%) at 140-60° till the evolution of hydrogen sulphide ceased (6-8 h) to afford **3** (Scheme I, Table I). For **3d** the characteristic frequencies appeared at 3010, 2981, 1686, 1604 and 823 cm^{-1} in its IR spectrum. PMR spectrum (DMSO-d_6) at 300 MHz showed signals at δ 5.4 (s, 2H, NH_2), 7.55-7.85 (2d, J = 9Hz Ar-H) and 13.2 (s, 1H, due to iminol form). ^{13}C NMR (DMSO-d_6) showed signals at 133 (-C=N), 167 (>C=O, amido form) and 128-132 ppm (Ar-H).

Compound **3** on treatment with aromatic aldehydes gave the corresponding Schiff bases **4** (Table I). For **4a**, IR (KBr) spectrum showed main absorption bands at 3051 (NH) and 1685 (broad > C=O). PMR (DMSO-d_6) at 270 MHz exhibited signals at δ 7.5 and 7.99 (2d, J = 9 Hz) for aromatic protons and a broad peak at 13.2 for -OH (tautomeric). ^{13}C NMR in DMSO-d_6 showed signals at 138 (>C=N carbon), 167 (carbonyl carbon) and 128 to 132 (aromatic carbons).

Compound **2** and phenylhydrazine when heated at 140-60° afforded **5** (Scheme I). The spectral characteristics and physical data are reported in



Scheme I

Table I.

4-Substituted-thiosemicarbazides **6** in alc NaOH were treated with a dry stream of carbon oxysulphide to obtain sodium β-(N-arylthiocarbamyl) thiocarbazinates **7** which were then cyclised in the presence of alc NaOH (8%) to give 5-arylamino-2-mercapto-1,3,4-oxadiazoles **8** (Scheme II, Table I). For **8a**, IR (KBr) spectrum showed bands at 1685, 1068, 1024, and 1001 cm^{-1}. PMR (DMSO-d_6) at 270 MHz exhibited signals at δ 3.5 (s, 1H, NH-Ph), 6.9-7.8 (m, 5H, aromatic), 9.8 (ring NH of tautomeric thioamido form). The signals at δ 3.5 and 9.8 were exchangeable with D_2O.

Compound **8** on reaction with benzyl chloride in the presence of alc NaOH (8%) gave the corresponding 3-benzylmercapto-4-substituted-1,2,4-triazolin-5-one **9** (Scheme II, Table I) by isomerisation. For **9a**, IR spectrum (KBr) spectrum exhibited characteristic carbonyl frequency at 1701 cm^{-1}. PMR spectrum (DMSO-d_6) at 270 MHz exhibited signals at δ 4.1 (s, 2H, S-CH_2), 7-7.5 (m, 10H, aromatic) and 8.3 (s, 1H, NH-CO) (exchangeable with D_2O).

Experimental Section

Melting points are uncorrected. The IR spectra (KBr) were recorded on a Perkin-Elmer FTIR 257 spectrometer and ^1H and ^{13}C NMR spectra on a Varian VXR 300S (300 MHz) and Brucker WH-270 MHz (67.89 MHz) FTNMR spectrometer respectively, using DMSO-d_6 as solvent. Carbon oxysulphide[10], acid hydrazides[11] **1** and thiosemicarbazides were prepared by known methods.

Potassium β-acyl thiocarbazinates 2. A steady stream of carbon oxysulphide was passed through a mixture of acid hydrazide (0.05 mole) and KOH

Table 1— Physical and spectral data of compounds **3, 4, 5, 7, 8 and 9**

Compd	R	R′	Mol formula	m.p. (°C)	Yield (%)	IR (ν_{max} in cm^{-1})
3a	C_6H_5	—	$C_8H_8N_4O$	238 (238)	68	3280, 3125, 1715, 1690, 1490, 785, 700
3b	*p*-CH_3-C_6H_4	—	$C_9H_{10}N_4O$	268 (268)	62	3260, 3120, 1715, 1690, 1490, 830
3c	*p*-$CH_3OC_6H_4$	—	$C_9H_{10}N_4O_2$	265 (263)	60	3300, 2982, 1686, 1604, 1465, 823
3d	*p*-Cl-C_6H_4	—	$C_8H_7ClN_4O$	234 (232)	78	3010, 2981, 1686, 1604, 1465,, 823
3e	*p*-OH-C_6H_4	—	$C_8H_8N_4O_2$	245	62	3315, 3194, 1700, 1615, 1467, 765
3f	4-Pyidyl	—	$C_7H_7N_5O$	274	57	3290, 3193, 1718, 1605, 1487, 829
3g	C_6H_5-O-CH_2	—	$C_9H_{10}N_4O_2$	83	77	3426, 3207, 3019, 2922, 1701, 1592, 1496, 1438, 1233, 756
3h	*p*-CH_3-C_6H_4O-CH_2	—	$C_{10}H_{12}N_4O_2$	129	76	3420, 3020, 2920, 1710, 1590, 1510, 1440, 1240, 770
3i	O-$CH_3C_6H_4$O-CH_2	—	$C_{10}H_{12}N_4O_2$	156	72	3425, 3020, 2917, 1738, 1594, 1498, 1453, 1242, 757
3j	*p*-Cl-C_6H_4O-CH_2	—	$C_9H_9ClN_4O_2$	149	81	3420, 3020, 2915, 1730, 1595, 1490, 1430, 1240, 710
3k	4-Cl(3-CH_3)-C_6H_3O-CH_2	—	$C_{10}H_{11}ClN_4O_2$	160	80	3441, 3022, 2924, 1736, 1616, 1477, 1245, 753
3l	*p*-Cl-C_6H_4S-CH_2	—	$C_9H_9ClN_4OS$	223	71	3325, 3219, 3040, 2916, 1733, 1695, 1475, 1305, 811
3m	C_6H_5S-CH_2	—	$C_9H_{10}N_4OS$	204	58	—
3n	4-Br-C_6H_4S-CH_2	—	$C_9H_9BrN_4OS$	257	64	—
4a	*p*-ClC_6H_4	C_6H_5	$C_{15}H_{11}ClN_4O$	248 (248)	67	3051, 2983, 1685, 1581, 1491, 1585, 850
4b	*p*-ClC_6H_4	foryl	$C_{13}H_9ClN_4O_2$	258	71	3050, 2981, 1684, 1589 1490, 848
4c	*p*-ClC_6H_4	*p*-OHC_6H_4	$C_{15}H_{11}ClN_4O_2$	281	68	3050, 2980, 1684, 1589
4d	*p*-$CH_3OC_6H_4$	C_6H_5	$C_{16}H_{14}N_4O_3$	271	61	3050, 2981, 1687, 1604, 1511, 843
4e	*p*-$CH_3OC_6H_4$	*o*-OHC_6H_4	$C_{16}H_{14}N_4O_3$	271	61	3180, 2982, 1683, 1604, 1513, 843
4f	*p*-$CH_3OC_6H_4$	*p*-$CH_3OC_6H_4$	$C_{17}H_{16}N_4O_3$	288	70	—
4g	*p*-$CH_3OC_6H_4$	furyl	$C_{14}H_{12}N_4O_3$	209	53	—
4h	C_6H_5	C_6H_5	$C_{15}H_{12}N_4O$	227 (227)	68	—
4i	C_6H_5	*o*-OHC_6H_4	$C_{15}H_{12}N_4O_2$	251	71	—
4j	C_6H_5	furyl	$C_{13}H_{10}N_4O_2$	218	59	—
4k	*p*-$CH_3C_6H_4$	C_6H_5	$C_{16}H_{14}N_4O$	203 (203)	81	—
4l	*p*-$CH_3C_6H_4$	*o*-OH-C_6H_4	$C_{16}H_{14}N_4O_2$	214	78	—
4m	*p*-$CH_3C_6H_4$	furyl	$C_{14}H_{12}N_4O_2$	222	65	—
4n	*p*-$CH_3C_6H_4$	*p*-$CH_3OC_6H_4$	$C_{17}H_{16}N_4O_2$	217	71	—
4o	*p*-OHC_6H_4	C_6H_5	$C_{15}H_{12}N_4O_2$	259	72	—
4p	*p*-OHC_6H_4	*o*-OHC_6H_4	$C_{15}H_{12}N_4O_3$	261	65	—
4q	*p*-OHC_6H_4	furyl	$C_{13}H_{10}N_4O_3$	222	59	—
4r	*p*-OHC_6H_4	*p*-$CH_3OC_6H_4$	$C_{16}H_{14}N_4O_3$	234	68	—
4s	*o*-OHC_6H_4	C_6H_5	$C_{15}H_{12}N_4O_2$	268	67	—
4t	*o*-OHC_6H_4	*o*-OHC_6H_4	$C_{15}H_{12}N_4O_3$	279	71	—

Contd.

Table 1— Physical and spectral data of compounds **3, 4, 5, 7, 8** and **9**—*Contd*

Compd	R	R′	Mol formula	m.p. (°C)	Yield (%)	IR (ν_{max} in cm^{-1})
4u	*o*-OHC_6H_4	furyl	$C_{11}H_{10}N_4O_3$	238	55	—
4v	*o*-OHC_6H_4	*p*-$CH_3OC_6H_4$	$C_{16}H_{14}N_4O_3$	251	68	—
4w	C_6H_5O-CH_2	C_6H_5	$C_{16}H_{14}N_4O_2$	227	70	3010, 2918, 1734, 1703, 1597, 1498, 1233, 1093, 834, 754
4x	C_6H_5O-CH_2	*o*-OHC_6H_4	$C_{16}H_{14}N_4O_3$	251	68	3402, 3011, 2919, 1734, 1703, 1597, 1498, 1234, 1093, 834, 755
4y	C_6H_5O-CH_2	*p*-$CH_3OC_6H_4$	$C_{17}H_{16}N_4O_3$	269	61	3011, 2919, 1734, 1704, 1597, 1498, 1235, 1094, 834, 755
4z	C_6H_5O-CH_2	furyl	$C_{14}H_{10}N_4O_3$	218	65	3011, 2919, 1734, 1704, 1597, 1498, 1235, 1094, 834, 755
4a′	*p*-$CH_3C_6H_4O$-CH_2	C_6H_5	$C_{17}H_{16}N_4O_2$	203	72	3020, 2923, 1735, 1702, 1612, 1436, 1236, 811
4b′	*p*-$CH_3C_6H_4O$-CH_2	*o*-OHC_6H_4	$C_{17}H_{16}N_4O_3$	214	64	3031, 2919, 1732, 1703, 1615, 1435, 1237, 813
4c′	*p*-$CH_3C_6H_4O$-CH_2	*p*-$CH_3OC_6H_4$	$C_{18}H_{18}N_4O_3$	271	68	3020, 2921, 1735, 1709, 1597, 1425, 1243, 798
4d′	*p*-$CH_3C_6H_4O$-CH_2	furyl	$C_{15}H_{14}N_4O_3$	222	59	3031, 2921, 1732, 1703, 1615, 1437, 1238, 813
4e′	*p*-ClC_6H_4O-CH_2	C_6H_5	$C_{16}H_{13}ClN_4O_2$	251	78	3020, 2910, 1735, 1706, 1594, 1429, 1235, 819,
4f′	*p*-ClC_6H_4O-CH_2	*o*-OHC_6H_4	$C_{16}H_{13}ClN_4O_3$	259	67	3423, 3020, 2910, 1735, 1708, 1594, 1428, 1234, 819
4g′	*p*-ClC_6H_4O-CH_2	*p*-$CH_3OC_6H_4$	$C_{16}H_{14}ClN_4O_3$	234	69	3080, 2911, 1708, 1736, 1894, 820 1736, 1429
4h′	*p*-ClC_6H_4O-CH_2	furyl	$C_{14}H_{11}ClN_4O_3$	212	64	3020, 2911, 1732, 1706, 1594, 1428, 1234, 820
4i′	*p*-Cl(3-CH_3)C_6H_3O-CH_2	C_6H_5	$C_{17}H_{15}ClN_4O_2$	217	74	3430, 3110, 2920, 1735, 1710, 1570, 1428, 1244, 870
4j′	*p*-Cl(3-CH_3)C_6H_3O-CH_2	*o*-OHC_6H_4	$C_{17}H_{15}ClN_4O_3$	222	69	3430, 3020, 2910, 1735, 1710, 1575, 1430, 1245, 870
4k′	*p*-Cl(3-CH_3)C_6H_3O-CH_2	*p*-$CH_3OC_6H_4$	$C_{18}H_{17}ClN_4O_3$	271	70	3030, 2910, 1735, 1710, 1570, 1470, 1240, 870
4l′	*p*-Cl(3-CH_3)C_6H_3O-CH_2	furyl	$C_{15}H_{13}ClN_4O_3$	261	62	3050, 2921, 1735, 1709, 1571, 1425, 1243, 867
5a	C_6H_5		$C_{14}H_{12}N_4O$	242 (245)	60	3223, 3084, 1717, 1600, 1497, 742, 687
5b	*p*-ClC_6H_4		$C_{14}H_{11}ClN_4O$	253	72	3265, 3052, 1728, 1657, 1498, 759, 687
5c	*p*-OHC_6H_4		$C_{14}H_{12}N_4O_2$	284	58	3326, 3071, 1628, 1492, 760, 653
5d	$C_6H_5OCH_2$		$C_{15}H_{14}N_4O_2$	192	61	3309, 2908, 1666, 1620, 1491, 722, 662
5e	*p*-$ClC_6H_4OCH_2$		$C_{15}H_{13}ClN_4O_2$	148	68	3253, 2937, 1645, 1620, 1492, 759, 651
5f	$C_6H_5SCH_2$		$C_{15}H_{14}N_4SO$	159	52	3331, 2913, 1660, 1607, 1476, 742, 675
5g	*p*-$ClC_6H_4SCH_2$		$C_{15}H_{13}ClN_4SO$	162	50	3200, 2908, 1664, 1578, 1481, 756, 684

Contd.

Table 1— Physical and spectral data of compounds **3, 4, 5, 7, 8** and **9**—*Contd*

Compd	R	R′	Mol formula	m.p. (°C)	Yield (%)	IR (ν_{max} in cm^{-1})
5h	$4\text{-}BrC_6H_9SCH_2$		$C_{15}H_{13}BrN_4SO$	179	48	—
7a	C_6H_5		$C_8H_8N_3NaOS_2$	284 (286)	58	3350, 3260, 3160, 1595, 1145
7b	$p\text{-}CH_3C_6H_4$		$C_9H_{10}N_3NaOS_2$	272 (274)	57	3580, 3280, 3165, 1625, 1155
7c	$p\text{-}ClC_6H_4$		$C_8H_7ClN_3NaO_2S_2$	298 (300)	62	3585, 3280, 3170, 1620, 1150
7d	$p\text{-}CH_3OC_6H_4$		$C_9H_{10}N_3NaO_2S_2$	292 (2294)	63	3520, 3244, 3159, 1592, 1156
8a	C_6H_5		$C_8H_7N_3OS$	142	89	3320, 1685, 1086, 1024, 1002
8b	$p\text{-}CH_3C_6H_4$		$C_9H_9N_3OS$	174	77	3344, 1649, 1274, 1068, 1024
8c	$p\text{-}ClC_6H_4$		$C_8H_6ClN_3OS$	148	80	3315, 1685, 1251, 1016
8d	$p\text{-}CH_3OC_6H_4$		$C_9H_9N_3O_2S$	189	69	3265, 1594, 1240, 1029
9a	C_6H_5		$C_{15}H_{13}N_3OS$	160	60	3139, 1701, 1280
9b	$p\text{-}CH_3C_6H_4$		$C_{16}H_{15}N_3OS$	190	61	3154, 1704, 1268
9c	$p\text{-}ClC_6H_4$		$C_{15}H_{12}ClN_3OS$	202	63	3156, 1704, 1279
9d	$p\text{-}CH_3OC_6H_4$		$C_9H_7N_4O_2S_2$	211	68	—

All compounds gave satisfactory C, H and N analyses.

3a: PMR: 11.84 (s, 1H, OH), 7.41-8.08 (m, 5H, aromatic), 4.80 (s, 2H, NH_2) at 90 MHz; ^{13}C NMR: 155 (>C=O, amido form), 145.63 (>C=N), 127-129.59 (aromatic carbons).

3b: PMR: 7.21-7.9 (4H, aromatic), 3.67 (s, 2H, NH_2) at 60 MHz.

3c: PMR: 11.9 (s, 1H, OH), 6.9-7.9 (m, 4H, aromatic), 5.4 (s, 2H, NH_2), 3.8 (s, 3H, OCH_3) at 300 MHz: ^{13}C NMR: 168 (>C=O, amido form), 163 (>C=N), 114-132 (aromatic carbons), 56 (CH_3O-).

3d: PMR: 13.2 (s, 1H, OH), 7.55-7.85 (m, 4H, aromatic), 5.4 (s, 2H, NH_2), at 300 MHz; ^{13}C NMR: 167 (>C=O, amido form), 138 (>C=N), 125-132 (aromatic carbons).

3e: PMR: 9.9 (s, 1H, OH), 9.4 (s, 1H, NH), 6.7-7.7 (m, 4H, aromatic), 5.3 (s, 2H, NH_2); ^{13}C NMR: 166 (>C=O, amido form), 160 (>C=N), 115-130 (aromatic carbons).

3g: PMR: 7.28-6.94 (m, 5H, aromatic), 7.8 (NH-C=O), 4.57 (s, 2H, CH_2), 4.8 (s, 2H, NH_2).

3h: PMR: 7.11-6.71 (m, 4H, aromatic), 8.04 (NH-C=O), 4.81 (s, 2H, NH_2), 4.53 (s, 2H, -O-CH_2), 2.24 (s, 3H, CH_3) at 90 MHz.

3I: PMR: 11.70 (s, 1H, NH), 7.2-6.75 (m, 4H, aromatic), 4.9 (s, 2H, NH_2), 4.6 (s, 2H, -OCH_2), 2.2 (s, 3H, -CH_3) at 300 MHz.

3j: PMR: 7.1-6.8 (m, 4H, aromatic), 7.7 (O=C-NH), 5.2 (s, 2H, NH_2), 4.4 (s, 2H, -OCH_2) at 60 MHz.

3k: PMR: 7.26-6.66 (m, 3H, aromatic), 7.91 (NH-NH-C=O), 5.2 (s, 2H, NH_2), 4.56 (s, 2H, -OCH_2), 2.31 (s, 3H, -CH_3) at 90 MHz.

3l: PMR: 11.5 (s, 1H, NH-C=O), 7.44-7.36 (m, 4H, aromatic), 5.2 (s, 2H, NH_2), 4.1 (s, 2H, -S-CH_2) at 300 MHz.

3m: PMR: 11.4 (s, 1H, NH-C=O), 7.56-7.19 (m, 5H, aromatic), 5.2 (s, 2H, NH_2), 4.1 (s, 2H, S-CH_2) at 300 MHz.

5a: PMR: 10.5 (s, 1H, OH), 9.1 (s, 1H, NH-C=O), 7.4-8 (m, 16H, aromatic) at 270 MHz.
^{13}C NMR: 167 (C=O), 154, 147 (-C=N, cyclic), 112-133 (aromatic carbon).

5f: PMR: 10.3 (s, 1H, OH), 9.4 (s, 1H, NH-C=O), 7.2-7.4 (m, 10H, aromatic), 3.7 (s, 2H, S-CH_2) at 270 MHz: ^{13}C NMR: 167 (C=O), 136 (-C=N), 126-132 (aromatic carbons), 235 (-CH_2).

5g: PMR: 9.9 (s, 1H, OH), 8.9 (s, 1H, NH-C=O), 7.3-7.6 (m, 9H, aromatic), 3.7 (s, 2H, S-CH_2) at 270 MHz; ^{13}C NMR: 167 (C=O), 135 (-C=N), 128-133 (aromatic carbons) and 35 (-CH_2).

8a: PMR: 3.5 (s, 1H, NH), 6.9-7.8 (m, 5H, aromatic), 9.8 (NH, tautomeric).

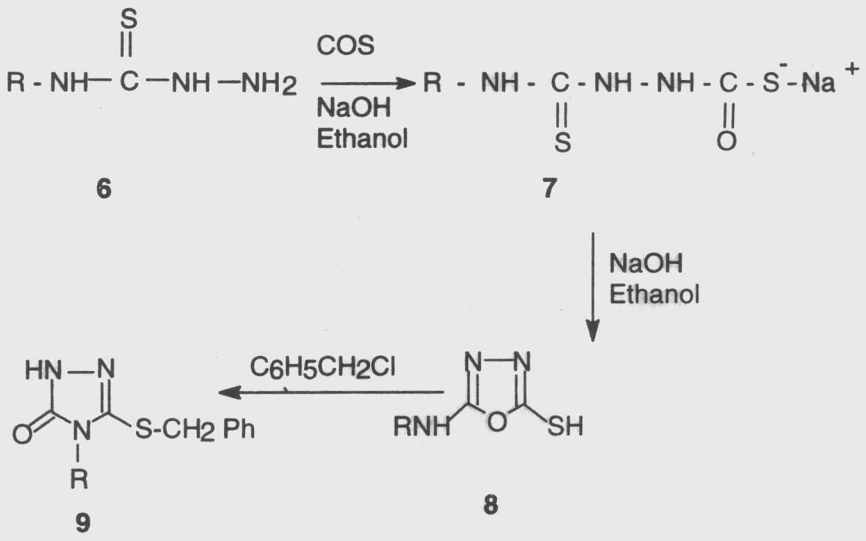
8b: PMR: 2.3 (s, 3H, Aryl-CH_3), 7.1-7.7 (m, 4H, aromatic), 9.7 (s, 1H, HN-C=O).

9a: PMR: 8.3 (s, 1H, NH-C=O), 7-7.5 (m, 10H, aromatic), 4.1 (s, 2H, S-CH_2).

(0.075 mole) in ethyl alcohol (30 mL) when **2** precipitated out in near quantitative yields. The product was washed with ether-acetone mixture (3:1) and was directly used for the synthesis.

Synthesis of 4-amino-3-substituted-1, 2, 4-triazolin-5-ones 3. A general procedure. 2 (0.05 mole) and hydrazine hydrate (99%, 0.15 mole) were heated together in an oil-bath at 140-60°C till the evolution of H_2S ceased (8 h). The reaction mixture was cooled and poured into ice-cold water (100 mL) and acidified with dil. HCl when **3** precipitated out. It was filtered, washed with cold water (2 × 50 mL) and recrystallized from aq. ethanol.

Schiff bases of 4-amino-substituted-1,2,4-triazolin-5-ones 4: A general procedure. A mixture of **3** (0.012 mole), aromatic aldehyde (0.012 mole) and sodium acetate (0.012 mole) was heated at reflux in ethanol (50 mL) for 4 h. The excess of ethanol was distilled out, the reaction mixture cooled and poured onto crushed ice. The product was filtered, washed with cold water and crystallized from ethanol-acetic acid mixture (1:1).



Scheme II

4-Amino-3-substituted-1,2,4-triazolin-5-ones 5: A general procedure. **2** (0.05 mole) and phenylhydrazine (0.05 mole) were heated together in an oil bath at 140-60°C till evolution of H_2S ceased (6 h). The reaction mixture was cooled and poured into ice-cold water (150 mL) and acidified with dil HCl. The solid thus obtained was filtered, washed with cold water (2 × 50 mL) and recrystallised from aq. ethanol (80%).

Sodium β-(N-arylthiocarbamyl) thiocarbazinates 7: A general procedure. Through the solution of 4-substituted thiosemicarbazide (0.02 mole) and NaOH (0.9 g) in abs ethanol (70 mL) was passed a dry stream of carbon oxysulphide for 3 h, under cooling. **7** thus obtained was filtered, washed with acetone and used directly for further reaction.

5-Substituted-2-mercapto-1,3,4-oxadiazoles 8. A solution of **7** (0.02 mole) in ethanol (20 mL) containing NaOH (0.02 mole) was heated at reflux till the evolution of H_2S was complete. It was cooled, poured onto crushed ice and acidified with dil. HCl to afford **8** which was crystallised from acetic acid.

3-Benzylmercapto-4-phenyl-1,2,4-triazolin-5-ones 9a. **8** (0.01 mole) and benzyl chloride (0.01 mole) were taken in 40 mL of alc NaOH (5%) and refluxed for 1.5 h. The solution was concentrated, cooled and then poured onto crushed ice. It was acidified with dil. HCl **9a** thus obtained was crystallised from ethanol.

Acknowledgement

Authors sincerely thank Director and Head of the Department of Chemistry, the Institute of Science, Bombay, for facilities and to RSIC, IIT, Bombay, Punjab University and the Director, Indian Institute of Science, Bangalore for PMR and ^{13}C NMR spectra. One of the authors (KSJ) thanks the Government of Maharashtra and University of Bombay for financial support.

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Indian Journal of Chemistry
Vol. 37B, April 1998, pp.358 - 364

New β-lactam derivatives: Synthesis and antibacterial activity of mono aryloxy-*s*-triazine derivatives of penicillin, cephalosporin, ampicillin and cephalexin

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Received 2 September 1997; accepted (revised) 17 February 1998

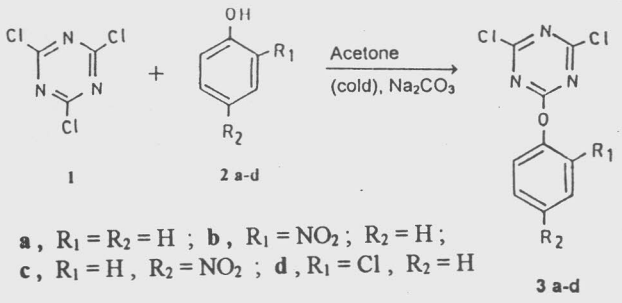
Mono aryloxy-*s*-triazine derivatives of penicillin, cephalosporin, ampicillin and cephalexin have been synthesised by the condensation of mono-substituted cyanuric chloride with 6-amino penicillanic acid (6-APA), 7-amino desacetoxy cephalosporanic acid (7-ADCA), ampicillin and cephalexin. The products have been tested for the evaluation of their antibacterial activity in terms of MIC values.

Most of the clinically used β-lactam antibiotics appear to be the side chain analogs of penicillin and cephalosporin. Large number of such analogs have been in use. But, in recent years, bacterial resistance to these antibiotics is found to be increasing at an alarming rate due to their overuse. As a result, successful treatment of bacterial infection is threatened. In order to overcome this problem there is an ever growing need to synthesise and try new antibiotics. In our own efforts in this direction, we have been preparing new penicillins[1] and cephalosporins with non-amide type linkage in the side chain. Synthesis of β-lactams with non-amide linkage has been reported earlier[2-8]. Among the first semisynthetic penicillins studied in man were aryloxyalkyl penicillins[9]; there was an improvement in the acid stability of these penicillins.

We were attracted by the structural features of 2,4,6-trichloro 1,3,5-triazine which are: (i) Its similarity to acyl and aroyl chlorides which when attached to 6-APA, 7-ADCA or 7-ACA may help in forming a stable acylenzyme which is crucial to the inhibition of transpeptidase activity thereby arresting the growth of bacteria; (ii) High reactivity of mono- and di-substituted cyanuric derivatives in nucleophilic substitution reactions.

Results and Discussion

The replacement reactions of cyanuric chloride with amines can be carried out stepwise so that



1 2 a-d 3 a-d

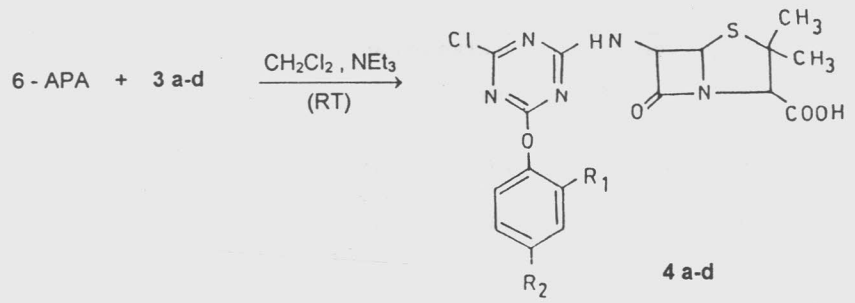
a, $R_1 = R_2 = H$; **b**, $R_1 = NO_2$; $R_2 = H$;
c, $R_1 = H$, $R_2 = NO_2$; **d**, $R_1 = Cl$, $R_2 = H$

Scheme I

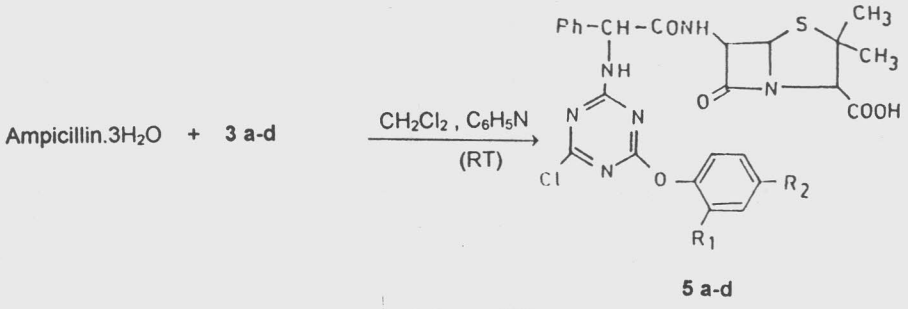
depending on the reaction conditions mono-, di- or tri-condensation products may be obtained. When the substituent is aryloxy the remaining two chlorine atoms are less deactivated and further condensation with 6-APA/7-ADCA and ampicillin/cephalexin is possible. Scheme I shows the synthesis of mono aryloxy-s-triazine derivatives and Schemes II-V show the synthesis of penicillin, cephalosporin, ampicillin and cephalexin derivatives. The structures of the products were confirmed by physical and spectral data (Tables I and II).

Antibacterial Activity

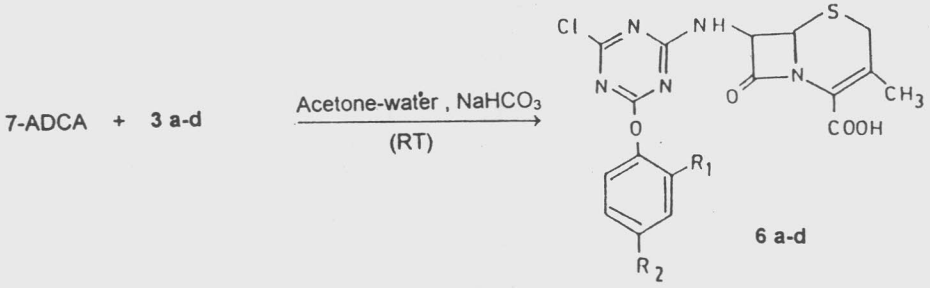
All the β-lactam derivatives were tested against gram +ve *S. aureus* bacteria and five gram -ve bacteria viz. *E.coli*, *K.pneumonie*, *S.typhi*, *P.aerugino* and *S. flexneri*. The standard agar plate diffusion technique described by Varma[10] was applied to determine the MIC value in mcg/mL at



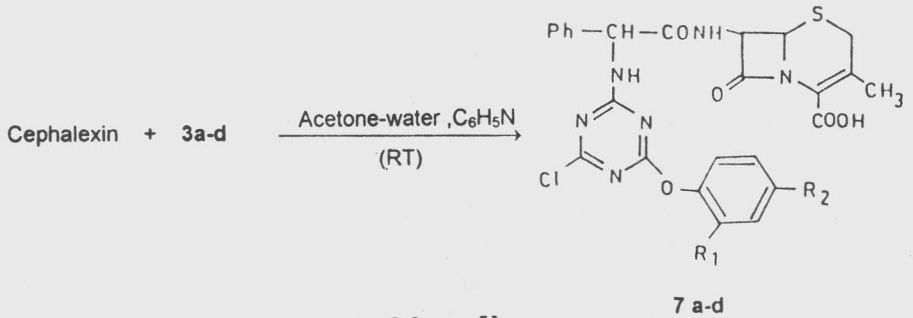
Scheme II



Scheme III



Scheme IV



Scheme V

which the growth of the bacterial cultures are completely suppressed. Ampicillin and cephalexin were used as standard antibiotics for comparison. The MIC values are given in Table III.

The unsubstituted phenoxy-*s*-triazine moiety when present in the side chain of 6-APA showed the highest activity (5 mcg/mL) against *S. aureus*. The activity was quite low when it was present as

Table I—physical data of the β-lactam derivatives **3a-d, 4a-d, 6a-d** and **7a-d**

Product	Yield (%)	m.p. (°C)	Mol. formula	Analysis (Calcd./Found) %				
				C	H	N	Cl	S
3a	88	229	$C_9H_5O\,N_3Cl_2$	44.60	2.06	17.35	29.34	—
				(44.61	2.0	17.29	29.31)	
3b	85	250(d)	$C_9H_4O_3N_4Cl_2$	37.63	1.39	19.51	24.74	—
				(37.60	1.33	19.49	24.7)	
3c	75	258(d)	$C_9H_4O_3N_4Cl_2$	37.63	1.39	19.51	24.74	—
				(37.61	1.41	19.53	24.75)	
3d	60	133	$C_9H_4ON_3Cl_3$	39.06	1.45	15.19	38.52	—
				(39.0	1.41	15.00	38.45)	
4a	28	151	$C_{17}H_{16}O_4N_5ClS$	48.40	3.79	16.61	8.40	7.59
				(48.5	3.61	16.71	8.30	7.65)
4b	12	195	$C_{17}H_{15}O_6N_6ClS$	43.73	3.21	18.00	7.59	6.86
				(43.82	3.14	17.5	7.50	6.81)
4c	49	167	$C_{17}H_{16}O_6N_6ClS$	43.73	3.21	18.00	7.59	6.86
				(43.82	3.19	17.91	7.49	6.80)
4d	50	172	$C_{17}H_{15}O_4N_5Cl_2S$	44.74	3.29	15.35	7.76	7.02
				(44.81	3.34	15.28	7.70	7.2)
5a	29	176	$C_{25}H_{23}O_5N_6ClS$	54.10	4.15	15.15	6.38	5.77
				(54.09	4.11	15.11	6.20	5.72)
5b	39	189	$C_{25}H_{22}O_7N_7ClS$	50.04	3.67	16.35	5.90	5.34
				(50.97	3.62	16.32	5.75	5.29)
5c	35	182	$C_{25}H_{22}O_7N_7ClS$	50.04	3.67	16.35	5.90	5.34
				(50.99	3.65	16.33	5.75	5.30)
5d	28	195	$C_{25}H_{22}O_5N_6Cl_2S$	50.93	3.73	14.26	6.01	5.43
				(50.89	3.79	14.30	5.90	5.46)
6a	34	149	$C_{17}H_{14}O_4N_5ClS$	48.63	3.34	16.69	8.44	7.63
				(48.58	3.39	16.74	8.3	7.59)
6b	25	144	$C_{17}H_{13}O_6N_6ClS$	43.92	2.80	18.08	7.62	6.89
				(43.88	2.76	17.98	7.56	6.94)
6c	20	189	$C_{17}H_{13}O_6N_6ClS$	43.92	2.80	18.08	7.62	6.89
				(43.87	2.85	18.12	7.50	6.86)
6d	25	170	$C_{17}H_{13}O_4N_5Cl_2S$	44.93	2.86	15.42	7.80	7.05
				(45.01	2.93	15.38	7.60	6.99)
7a	28	189	$C_{25}H_{21}O_5N_6Cl_2S$	54.30	3.80	15.20	6.41	5.79
				(54.27	3.77	15.18	6.30	5.83)
7b	24	196	$C_{25}H_{21}O_5N_6Cl_2S$	50.21	3.35	16.40	5.92	5.36
				(50.18	3.40	16.37	5.80	5.29)
7c	42	184	$C_{25}H_{20}O_7N_7ClS$	50.21	3.35	16.40	5.92	5.36
				(50.26	3.38	16.44	5.80	5.31)
7d	31	194	$C_{25}H_{20}O_5N_6Cl_2S$	51.15	3.41	14.32	6.03	5.46
				(51.20	3.48	14.28	5.90	5.41)

the α-amino substituent of ampicillin. This may be due to the incorporation of the α-carbon atom with aromatic ring resulting in low activity against gram +ve bacteria. The same substituent in α-amino of cephalexin showed better activity, 100 mcg/mL against *S. aureus*. This is in keeping with the fact that, the simple acylation of amino group in the side chain of cephalosporin C increases the activity against gram +ve bacteria.

The nitro- and chloro-phenoxy-*s*-triazine derivatives of penicillins and cephalosporins showed better activity against *S. aureus* when these electron withdrawing groups were present in the *ortho* position.

The phenoxy-*s*-triazine derivatives of 6-APA, 7-ADCA, ampicillin and cephalexin showed better Activity against *S. typhi* and *S. flexneri*. The *o*-chloro-phenoxy-*s*-triazine derivatives of penicillin and cephalosporin were more active against gram –ve bacteria than the *o*-nitro and -nitrophenoxy-*s*-

Table II—spectral data of the β-lactam derivatives **3a-d, 4a-d, 5a-d, 6a-d** and **7a-d**

Product	λ_{max} (EtOH, nm)	IR (KBr) cm^{-1}	^1H NMR (DMSO-d_6) δ (ppm)
3a	256	1210 (C-O-C); 840 (C_3N_3Cl); 1540 (C=N, cycl. conj.)	—
3b	240	1210 (C-O-C); 840(C_3N_3Cl); 1540 (C=N, cycl. conj.)	—
3c	260	1210 (C-O-C); 810(C_3N_3Cl); 1540 (C=N, cycl. conj.)	—
3d	245	1210 (C-O-C); 810(C_3N_3Cl); 1540 (C=N, cycl. conj.)	—
4a	240	1760 (β lactam C=O); 1680 (COOH); 1210 (C-O-C); 840 (C_3N_3Cl); 1540 (C=N, cycl. conj.); 3200 (NH)	1.15 (3H, t, 2α - CH_3) 1.45 (3H,m,2β - CH_3) 4.0 (1H,q,3-H); 5.4(1H,d,5-H) 5.7 (1H,dd,6-H); 4.9(1H,d,NH) 7.4-8.1 (4H, m, Ar-H)
4b	245	1740 (β lactam C=O); 1700 (COOH); 1220 (C-O-C); 800 (C_3N_3Cl); 1520 (C=N, cycl. conj.); 3200 (NH)	1.15 (3H, t, 2α - CH_3), 1.45 (3H,m,2β - CH_3), 4.0 (1H,q,3-H); 5.4(1H,d,5-H), 5.7 (1H,dd,6-H); 4.9(1H,d,NH), 7.4-8.1 (4H, m, Ar-H).
4c	259	1750 (β lactam C=O); 1700 (COOH); 1200 (C-O-C); 820 (C_3N_3Cl); 1540,1560 (C=N, cycl. conj.); 3200 (NH)	1.15 (3H, t, 2α - CH_3), 1.45 (3H,m,2β - CH_3), 4.0 (1H,q,3-H); 5.4(1H,d,5-H), 5.7 (1H,dd,6-H); 4.9(1H,d,NH), 7.4-8.1 (4H, m, Ar-H).
4d	250	1755 (β lactam C=O); 1680 (COOH); 1210 (C-O-C); 840 (C_3N_3Cl); 1560 (C=N, cycl. conj.); 3100 (NH)	1.15 (3H, t, 2α - CH_3), 1.45 (3H,m,2β - CH_3), 4.0 (1H,q,3-H); 5.4(1H,d,5-H), 5.7 (1H,dd,6-H); 4.9(1H,d,NH), 7.4-8.1 (4H, m, Ar-H).
5a	240	1780 (β lactam C=O); 1720 (side chain amide C=O); 1680 (COOH); 1210 (C-O-C); 840 (C_3N_3Cl); 1540 (C=N, cycl. conj.);	1.15 (3H, t, 2α - CH_3), 1.45 (3H,m,2β - CH_3), 4.0 (1H,q,3-H); 5.4 (1H,d,5-H), 5.7(1H,dd,6-H); 6.2 (1H,s,CONH), 4.2 (1H,d,Ph-CH); 4.9 (1H,d,NH), 7.4-8.1 (4H, m, Ar-H).
5b	250	1780 (β lactam C=O); 1720 (side chain amide C=O); 1680 (COOH); 1210 (C-O-C); 840 (C_3N_3Cl); 1540 (C=N, cycl. conj.);	1.15 (3H, t, 2α - CH_3), 1.45 (3H,m,2β - CH_3), 4.0 (1H,q,3-H); 5.4 (1H,d,5-H), 5.7(1H,dd,6-H); 6.2 (1H,s,CONH), 4.2 (1H,d,Ph-CH); 4.9 (1H,d,NH), 7.4-8.1 (4H, m, Ar-H).
5c	260	1780 (β lactam C=O); 1720 (side chain amide C=O); 1680 (COOH); 1210 (C-O-C); 840 (C_3N_3Cl); 1540 (C=N, cycl. conj.);	1.15 (3H, t, 2α - CH_3), 1.45 (3H,m,2β - CH_3), 4.0 (1H,q,3-H); 5.4 (1H,d,5-H), 5.7(1H,dd,6-H); 6.2 (1H,s,CONH), 4.2 (1H,d,Ph-CH); 4.9 (1H,d,NH), 7.4-8.1 (4H, m, Ar-H).

(Contd)

Table II—Spectral data of the β-lactam derivatives **3a-d, 4a-d, 5a-d, 6a-d** and **7a-d**—*Contd*

Product	λ_{max} (EtOH, nm)	IR (KBr) cm^{-1}	^1H NMR (DMSO-d_6) δ (ppm)
5d	270	1780 (β lactam C=O); 1720 (side chain amide C=O); 1680 (COOH); 1210 (C-O-C); 840 (C_3N_3Cl); 1540 (C=N, cycl. conj.);	1.15 (3H, t, 2α - CH_3), 1.45 (3H,m,2β - CH_3), 4.0 (1H,q,3-H); 5.4 (1H,d,5-H), 5.7(1H,dd,6-H); 6.2 (1H,s,CONH), 4.2 (1H,d,Ph-CH); 4.9 (1H,d,NH), 7.4-8.1 (4H, m, Ar-H).
6a	282	1770 (β lactam C=O); 1650 (carboxylic C=O); 1600 (C=C); 800 (C_3N_3Cl); 1540, 1560 (C=N, cycl. conj.); 1220 (C-O-C)	3.2, 3.5 (2H, ABq, CH_2); 2.1 (3H,s,CH_3); 5.4 (1H,d,6-H); 5.8 (1H,q,7-H); 4.9(1H,NH); 7.4-8.1 (4H, m, Ar-H).
6b	280	1770 (β lactam C=O); 1640 (carboxylic C=O); 1680 (C=C); 810 (C_3N_3Cl); 1530 (C=N, cycl. conj.); 1220 (C-O-C)	3.3 (2H, ABq, CH_2); 2.0 (3H,s,CH_3); 5.45 (1H,d,6-H); 5.8 (1H,q,7-H); 5.1 (1H,NH); 7.5-8.2 (4H, m, Ar-H).
6c	279	1770 (β lactam C=O); 1650 (carboxylic C=O); 1600 (C=C); 800 (C_3N_3Cl); 1560 (C=N, cycl. conj.); 1220 (C-O-C)	3.3 (2H, ABq, CH_2); 2.1 (3H,s,CH_3); 5.4 (1H,d,6-H); 5.8 (1H,q,7-H); 5.1 (1H,d,NH); 7.5-8.1 (4H, m, Ar-H).
6d	281	1770 (β lactam C=O); 1650 (carboxylic C=O); 1600 (C=C); 850 (C_3N_3Cl); 1540, 1560 (C=N, cycl. conj.); 1220 (C-O-C)	3.4, 3.6 (2H, ABq, CH_2); 2.2 (3H,s,CH_3); 5.5 (1H,d,6-H); 5.8 (1H,q,7-H); 5.2 (1H,d,NH); 7.5-8.1 (4H, m, Ar-H).
7a	280	1750 (β lactam C=O); 1720 (side chain amide); 1600 (C=C); 1620 (COOH); 840 (C_3N_3Cl); 1560 (C=N, cycl. conj.); 1220 (C-O-C)	3.2, 3.5 (2H, ABq, CH_2); 2.1 (3H,s,CH_3); 5.4 (1H,d,6-H); 5.8 (1H,q,7-H); 6.2 (1H,d,CONH); 4.8 (1H,d,Ph-CH); 5.1(1H,d,NH); 7.4-8.1 (4H, m, Ar-H).
7b	275	1760 (β lactam C=O); 1700 (side chain amide); 1600 (C=C); 1620 (COOH); 830 (C_3N_3Cl); 1540 (C=N, cycl. conj.); 1220 (C-O-C)	3.2, 3.5 (2H, ABq, CH_2); 2.1 (3H,s,CH_3); 5.4 (1H,d,6-H); 5.8 (1H,q,7-H); 6.2 (1H,d,CONH); 4.8 (1H,d,Ph-CH); 5.1(1H,d,NH); 7.4-8.1 (4H, m, Ar-H).
7c	278	1765 (β lactam C=O); 1720 (side chain amide); 1600 (C=C); 1620 (COOH); 840 (C_3N_3Cl); 1540 (C=N, cycl. conj.); 1200 (C-O-C)	3.2, 3.5 (2H, ABq, CH_2); 2.1 (3H,s,CH_3); 5.4 (1H,d,6-H); 5.8 (1H,q,7-H); 6.2 (1H,d,CONH); 4.8 (1H,d,Ph-CH); 5.1(1H,d,NH); 7.4-8.1 (4H, m, Ar-H).
7d	282	1765 (β lactam C=O); 1720 (side chain amide); 1600 (C=C); 1620 (COOH); 840 (C_3N_3Cl); 1540 (C=N, cycl. conj.); 1200 (C-O-C)	3.2, 3.5 (2H, ABq, CH_2); 2.1 (3H,s,CH_3); 5.4 (1H,d,6-H); 5.8 (1H,q,7-H); 6.2 (1H,d,CONH); 4.8 (1H,d, Ph-CH); 7.4-8.1 (4H, m, Ar-H).

Table III—*In vitro* evaluation of the β-lactam derivatives (**4a-d, 5a-d, 6a-d** and **7a-d**) for anti-bacterial activity

Derivative	MIC (mcg/mL) species					
	S.aureus	*E.coli*	*K.pnemoniae*	*S.typhi*	*P.aeruginosa*	*S.flexneri*
4a	5	200	100	20	500	10
4b	50	500	200	50	500	400
4c	100	200	100	20	200	20
4d	50	100	50	50	200	400
5a	>500	100	50	40	500	100
5b	100	500	100	40	>500	200
5c	100	100	50	60	400	50
5d	50	50	40	50	200	100
6a	500	400	400	200	>500	200
6b	400	500	300	100	200	100
6c	400	400	300	200	>500	300
6d	500	500	400	500	>500	400
7a	100	200	200	100	200	100
7b	100	100	100	200	400	50
7c	200	50	200	50	500	50
7d	100	50	100	50	200	50
Std. 1	5	9	50-100	—	>200	11
Std. 2	0.016	0.5	>100	1	500	—

Std. 1—Cephalexin, **Std. 2**—Ampicillin

triazine derivatives. This may be due to the chlorine atom, which is a common observation.

Experimental Section

Mono aryloxy-*s*-triazines 3a-d. Cyanuryl chloride, sodium carbonate and appropriate phenol were taken in equimolar amounts and were dissolved in aq. acetone. The contents were cooled to −10°C and kept stirring for 3 hr. After completion of the reaction the products were purified and characterised as shown (Tables I and II).

Mono aryloxy-s-triazine derivatives of 6-APA 4a-d. To a suspension of 6-APA (2.5 mmoles) in dry dichloromethane (10 ml) kept at 0°C was added triethylamine (3.6 mmoles) dropwise to get clear solution. The compound 3a-d was then added in small portions and the reaction mixture was stirred for 2-4 h at RT. The progress of the reaction was monitored by TLC. At the end of the reaction the contents were acidified, extracted with ethyl acetate to get the product which was characterised (Tables I and II).

Mono aryloxy-s-triazine derivatives of ampicillin 5a-d. To a cold suspension of ampicillin trihydrate (1.24 mmoles) in dry dichloromethane (15 mL) was added pyridine (2.5 mmoles) to get clear solution. To this was added (1.24 mmoles) of the compound **3a-d** in small amounts followed by magnesium sulphate (1.24 mmoles) and the reaction mixture was stirred for 3 hr at RT. Product formation was followed on TLC. The contents were cooled to 9°C and acidified to *p*H 3 with 1*N* HCl. The product was recrystallised from methanol-water and characterised (Tables I and II).

Mono aryloxy-s-triazine derivatives of 7-ADCA 6a-d To a cold suspension of 7-ADCA (3 mmoles) in aq. acetone (10 mL) was added sodium bicarbonate solution dropwise untill *p*H 8 was obtained. To this was added (3 mmoles) of the compound **3a-d** in small portions. The reaction was stirred at RT for 5 hr. The progress of the reaction was monitored by TLC. The products separated on acidification were recrystallized from acetone-water and characterised as shown (Tables I and II).

Mono aryloxy-s-triazine derivatives of cephalexin 7a-d Cephalexin (2.15 mmoles) was taken in acetone (5 mL) and water (10 mL). To this was added pyridine (3.5 mmoles) dropwise with cooling and stirring to get a clear solution compound **3a-d** (2.15 moles) was added in small portions and the contents were stirred at RT for 4h. The progress of the reaction was monitored on TLC. The products were separated by acidification (*p*H 2) with dilute H_2SO_4 and recrystallised in Methanol-water and characterised as shown in Tables I and II.

Acknowledgement

The authors are grateful to the Lady Tata Memorial Trust, Bombay for the financial support to undertake the project.

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Indian Journal of Chemistry
Vol. 37B, April 1998, pp.365 - 369

Synthesis of antibacterial isocoumarins: Synthesis and antibacterial activity of 3-alkylisocoumarins and (*dl*)-3-alkyl-3,4-dihydroisocoumarins

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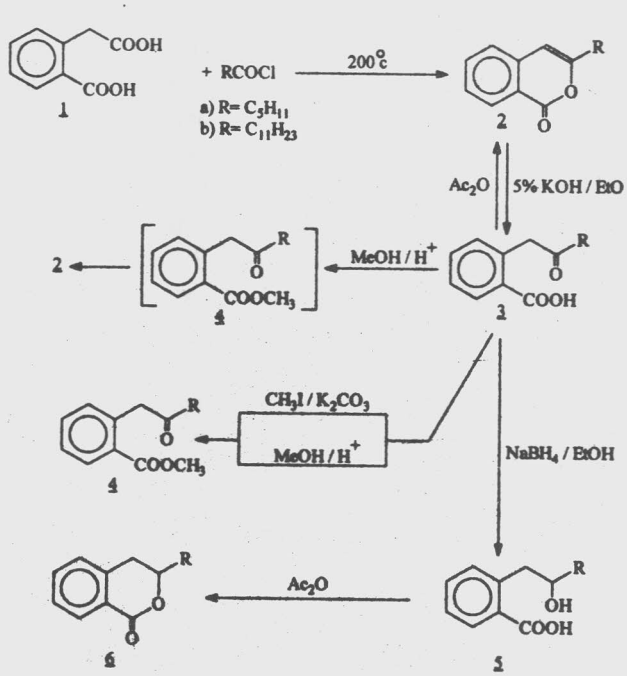
Received 26 March 1997; accepted (revised) 3 November 1997

3-Pentyl- and 3-undecylisocoumarins **2a,b** have been conveniently prepared in high yields by direct condensation of acyl chlorides with homophthalic acid. Alkaline hydrolysis of **2a,b** yields the corresponding ketoacids **3a,b**. The latter are reconverted to **2a,b** either by treatment with acetic anhydride or with slightly acidified methanol. Treatment of **3a,b** with methyl iodide or dry methanol in the presence of a catalytic amount of sulfuric acid affords the methyl keto-esters **4a,b**. (*dl*)-3,4-Dihydroisocoumarins **6a,b** are obtained by reduction of **3a,b** to the racemic hydroxyacids **5a,b** followed by cyclodehydration using acetic anhydride. The isocoumarins **2a,b** and dihydroisocoumarins **6a,b** have been examined *in vitro* for their antibacterial activity. Compounds **2a** and **6b** show significant activity comparable to the standard antibiotics.

Isocoumarins and 3,4-dihydroisocoumarins are the metabolites of a wide variety of fungi, bacteria, insects and higher plants[1,2] and exhibit a wide spectrum of biological activities such as antifungal, antihypertensive, antirheumatic and anticoagulant. However, there are only a few reports of the antibacterial action of isocoumarins like isocoumarinylpenicillin derivatives. In this article we report that simple 3-alkylisocoumarins and dihydroisocoumarins are quite effective against the pathogenic Gram positive and Gram negative bacteria.

3-Pentyl- and 3-undecyl-isocoumarins were prepared by the method of Nakajima *et al.*[3,4] involving the direct condensation of hexanoyl and dodecanoyl chlorides with homophthalic acid. The isocoumarins showed characteristic 1H singlet at δ 6.22 for C_4-H and the lactonic carbonyl absorptions at 1720 and 1715 cm^{-1}. Alkaline hydrolysis afforded the keto acids **3a,b**. In the ^1H NMR of these compounds 2H singlets at δ 4.02 and 4.03 and 1H exchangeable broad singlets at δ10.2 and 11.3 were observed. The carbonyl absorptions were observed at 1720 and 1680 cm^{-1}. Isocoumarins **2a,b** were obtained by refluxing the keto-acids **3a,b** with acetic anhydride. Following an already reported procedure[7], compounds **3a,b** were refluxed with dry acidic methanol for 8 hr to furnish the keto-esters **4a,b** as indicated by TLC. It may be pointed out that the work-up of this reaction mixture involved the use of sodium bicarbonate which might have hydrolyzed the keto-esters **4a,b** to the corresponding keto-acids **3a,b** which under the



work-up conditions were ocnverted to the lactones **2a,b** identical in all respect with those prepared earlier. Methylation of **3a,b** with excess of methyl iodide or with acidic dry methanol under reflux for 8 hr without using sodium bicarbonate in the work-up also yielded the methyl keto-esters **4a,b** which showed carbonyl absorption at 1720 cm^{-1} and a 3H singlet at δ 3.82. It is presumed that sodium borohydride reduction of **3a,b** afforded the corresponding hydroxyacids **5a,b** (not isolated) which under the direct influence of acetic anhydride yielded the racemic dihydroisocoumarins **6a,b**. The latter showed carbonyl absorption at 1720 cm^{-1} and the typical *ABX* pattern of C_3-H and C_4-H protons in ^1H PMR spectra. Thus, each of the C-4 protons showed a double doublet (δ 2.86-2.89 and 2.92-2.97 respectively). The protons of methylene groups adjacent to either side of the chiral center exhibited diastereotopic effect.

Antibacterial activity

The isocoumarins **2a,b** and dihydroisocoumarins **6a,b** were tested *in vitro* for their antibacterial activity against pathogenic Gram positive and Gram negative bacteria and the results are summarized in Table I. 3-Pentylisocoumarin **2a** showed activity against two Gram negative bacteria, *Staphylococcus aureus* and *Corynebacterium diphtheriae* comparable to the standard antibiotics Ampicillin and Amoxicillin. 3-Pentyldihydroisocoumarin **6a** and 3-undecyldihydroisocoumarin **6b** were found active against three Gram positive, *S. aureus, C. diphtheriae* and *Bacillus cesus*, and two Gram negative bacteria, *Escherichia coli* and *Salmonella typhi* in accordance with the general trend that the 3,4-dihydroisocoumarins are more potent than the corresponding isocoumarins.

Experimental Section

General. Melting points were determined using a MELTEMP MP-D apparatus and are uncorrected. IR spectra were recorded on a Hitachi 270-50 infrared spectrophotometer as KBr discs or as neat liquids. ^1H NMR (500 MHz) spectra were recorded in $CDCl_3$ on a Bruker AM-500 spectrometer using TMS as internal standard and EIMS on a MAT-112-S machine.

3-Pentylisocoumarin 2a. A mixture of homophthalic acid (2.0 g, 0.011 mole) and hexanoyl chloride (6.28 g, 0.0466 mole) was heated on an oil-bath at 200°C for 3 hr and then refluxed for 1 hr with methanol (20 mL) to convert the excess hexanoyl chloride into ester. The residue obtained after concentration was chromatographed over silica gel column using pet. ether (60-80°) as eluant affording **2a** as a yellow oil (1.5 g, 0.007 mole, 63%); IR (neat): 1720, 1665, 1615, 1590 cm^{-1}; ^1H NMR: δ 0.88 (3H, t, *J*=7.2 Hz, H-5′), 1.32 (4H, m, H-3′,4′), 1.68 (2H, p, *J*=7.4, 10.3 Hz, H-2′), 2.49 (2H, t, *J*=7.6 Hz, H-1′), 6.22 (1H, s, H-4), 7.32-7.33 (1H, dd, *J*=1.15, 7.8 Hz, H-5), 7.39-7.42 (1H, ddd, *J*=1.15, 7.5, 8.2 Hz, H-7), 7.62-7.65 (1H, ddd, *J*=1.35, 7.4, 8.7 Hz, H-6), 8.20-8.22 (1H, dd, *J*=1.25, 8.0 Hz, H-8); MS (70 eV): m/z 216 (100%) [M^+], 188 (5.48), 159 (4.43), 118 (44.37), 57 (78.56) [$C_{14}H_{16}O_2$: Calcd 216.1150; Found 216.1130 (MS)].

2-(2-Oxoheptyl)benzoic acid 3a. A solution of 3-pentylisocoumarin **2a** (0.5 g, 0.0024 mole) in etha-

Table I—Antibacterial activities of isocoumarins **2a,b** and 3,4-dihydroisocoumarins **6a,b**

Compd	Zone of inhibition (mm) 200 μg/100 mL				
	Staphylococcus aureus	*Corynebacterium diphtheriae*	*Bacillus cesus*	*Escherichia coli*	*Salmonella tryphi*
2a	9.0	2.0	—	—	—
2b	—	—	—	—	—
6a	—	—	—	—	—
6b	9.0	7.5	8.5	5.5	8.0
Ampicillin	12.0	—	8.5	15.0	11.0
Amoxicillin	10.0	10.5	9.0	16.0	14.0
Cfuroxime	—	15.5	*	18.5	8.0

— =Not screened; *=No inhibition of growth was observed at 100 μg/mL concentration.

nol (20 mL) and potassium hydroxide (5%, 40 mL) was refluxed for 4 hr. After cooling the reaction mixture, ethanol was removed under reduced pressure. Cold water (30 mL) was then added and the mixture acidified with dil hydrochloric acid and extracted with dichloromethane (2×15 mL). The extract was dried (Na_2SO_4), and the solvent removed on a rotary evaporator to give **3a** as a yellow oil (0.4 g, 0.0017 mole, 74%); IR (neat): 1720, 1680, 1605 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.9 (3H, t, *J*=7.0 Hz, H-7′), 1.32 (4H, m, H-5′,6′), 1.62 (2H, p, *J*=7.36, 14.73 Hz, H-4′), 2.47 (2H, t, *J*=7.38 Hz, H-3′), 4.02 (2H, s, H-1′), 7.18-7.20 (1H, dd, *J*=1.15, 7.8 Hz, H-3), 7.35-7.39 (1H, ddd, *J*=1.1, 7.7, 8.7 Hz, H-5), 7.50-7.54 (1H, ddd, *J*=1.4, 7.5, 8.9 Hz, H-4), 8.11-8.13 (1H, dd, *J*=1.32, 7.8 Hz, H-6), 10.2 (1H, br.s, D_2O exchangeable, COOH); MS (70 eV): m/z 234 (10.65%) [M^+], 216 (14.8) [M^+–H_2O], 163 (2.43), 135 (15.59), 118 (100), 99 (59.69), 71 (66.18) [$C_{14}H_{18}O_3$: Calcd 234.1243; Found 234.1243 (MS)].

3-Pentylisocoumarin 2a. Method A. Compound **3a** (30 mg, 0.13 mmole) was refluxed with acetic anhydride (0.7 mL, 6.8 mmole) for 12 hr. After cooling, the reaction mixture was poured into ice-water (20 mL) and extracted with ethyl acetate (2×5 mL). The extracts were combined, washed with sodium bicarbonate (2×5 mL, 5%) and water (2×10 mL), and the organic layer was dried with (Na_2SO_4) and concentrated to give **2a** as an oil (25 mg, 0.12 mmole, 89%); R_f value, mass, high resolution mass, IR and ^1H NMR spectral data were in good agreement with those of the already synthesized **2a**.

Method B. A solution of **3a** (150 mg, 0.64 mmole) in dry methanol (100 mL) and conc. sulfuric acid (as a catalyst) was refluxed for 8 hr. The reaction mixture was cooled, neutralized with solid sodium bicarbonate and filtered. The filtrate was rotatory evaporated to afford a yellow oil which was purified by preparative thin layer chromatography to furnish **2a** (120 mg, 0.56 mmole, 89%) as a bright yellow oil.

Methyl 2-(2-oxoheptyl)benzoate 4a. Method A. A mixture of **3a** (150 mg, 0.64 mmole), methyl iodide in excess and anhydrous potassium carbonate (1.0 g) in dry acetone (10 mL) was heated under reflux for 1 hr. The reaction mixture was filtered while hot, the cake washed with warm dry acetone (10 mL) and the solvent evaporated *in vacuo* leaving **4a** as a yellow oil (140 mg, 0.58 mmole, 90%); R_f value, mass, high resolution mass, IR and ^1H NMR spectral data were in good agreement with those of **4a** synthesized by Method B.

Method B. A solution of **3a** (150 mg, 0.64 mmole) in dry methanol (100 mL) and conc. sulfuric acid (two drops) was refluxed for 8 hr. Water (50 mL) was then added, methanol removed under reduced pressure and the reaction mixture extracted with ether (2×20 mL). The combined extract was dried (Na_2SO_4), the solvent removed under reduced pressure, and the residue purified by preparative thin layer chromatography to furnish **4a** as a yellow oil (91 mg, 0.37 mmole, 57%); IR (neat): 1720, 1685 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.89 (3H, t, *J*=7.2 Hz, H-7′), 1.34 (4H, m, H-5′,6′), 1.69 (2H, p, *J*=7.4, 15 Hz, H-4′), 2.50 (2H, t, *J*=7.85 Hz, H-3′), 3.82 (3H, s, OMe), 4.06 (2H, s, H-1′), 7.32-7.34 (1H, dd, *J*=3.2, 7.05 Hz, H-3), 7.41-7.44 (1H, ddd, *J*=1.15, 7.5, 8.2 Hz, H-5), 7.66-7.63 (1H, ddd, *J*=1.35, 3.8, 7.75 Hz, H-4), 8.24-8.22 (1H, ddd, *J*=0.65, 1.25, 8Hz, H-6); MS (70 eV): m/z 248 (5.10%) [M^+], 217 (3.39), 216 (100), 192 (8.32), 118 (16.3) [$C_{15}H_{20}O_3$: Calcd 248.1412; Found 248.1420 (MS)].

(*dl*)-3,4-Dihydro-3-pentylisocoumarin 6a. Compound **3a** (150 mg, 0.64 mmole) was heated under reflux with sodium borohydride (0.15 g) in abs. ethanol (15 mL) for 4 hr. Ethanol was then rotatory evaporated and the residue diluted with cold water and acidified with dil. sulfuric acid to give a precipitate which was extracted with ethyl acetate (2×10 mL). The solvent was evaporated to leave **5a** as an oil (0.12 g). This crude compound was dissolved in acetic anhydride (1 mL) and heated under reflux for 2 hr. The reaction mixture was then cooled, and water (10 mL) was added. The oil which separated on stirring was extracted with dichloromethane (2×5 mL). The extracts were combined, treated with sodium bicarbonate (2×5 mL, 5%), washed with water, dried over sodium sulfate (anhydrous) and filtered. The filtrate was stripped off solvent on a rotary evaporator to leave **6a** as an oil (100 mg, 0.46 mmole, 71%); IR (neat):

1720, 1615 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.88(3H, t, *J*=7.05 Hz, H-5′), 1.3 (4H, m, H-3′,4′), 1.5 (2H, p, *J*=7.2, 14.3 Hz, H-2′), 1.66-1.72 (1H, m, H-1′), 1.82-1.89 (1H, m, H-1′), 2.86-2.89 (AB pattern, 1H, d, d, J_{vic}=3.6, J_{gem}=16.25 Hz, H-4), 2.92-2.97 (*AB* pattern, 1H, d, d, J_{vic}=11.0, J_{gem}=16.2 Hz, H-4), 4.52-4.47 (1H, m, H-3), 7.20-7.22 (1H, dd, *J*=1.1, 7.55 Hz, H-5), 7.32-7.37 (1H, dd, *J*=5.75, 13.3 Hz, H-7), 7.48-7.51 (1H, ddd, *J*=1.4, 7.55, 8.95 Hz, H-6), 8.05-8.07 (1H, dd, *J*=1.25, 7.8 Hz, H-8); MS (70 eV): m/z 218 (5.32%) [M^+], 217 (31.47), 146 (76.49), 118 (100), 91 (81.18) [$C_{14}H_{18}O_2$: Calcd 218.1307; Found 218.1317 (MS)].

3-Undecylisocoumarin 2b. A mixture of homophthalic acid (2.0 g, 0.011 mole) and dodecanoyl chloride (16.2 g, 0.047 mole) was heated in an oil-bath at 200°C for 3 hr. The residue was purified by column chromatography over silica gel using pet. ether (60-80°) as eluant to afford **2b** (2.8 g, 9.3 mmole, 87%) as a semi-so9lid; IR (neat): 1715, 1660, 1610 cm^{-1}, ^1H NMR ($CDCl_3$): δ 0.86 (3H, t, *J*=7.05 Hz, H-11′), 1.24-1.37 (16H, m, H-3′-10′), 1.57-1.63 (2H, p, *J*=7.4, 14.75 Hz, H-2′), 2.49-2.52 (2H, t, *J*=7.75 Hz, H-1′), 6.23 (1H, s, H-4), 7.32-7.34 (1H, dd, *J*=1.15, 7.6 Hz, H-5), 7.41-7.44 (1H, ddd, *J*=1.1, 7.65, 8.75 Hz, H-7), 7.63-7.66 (1H, ddd, *J*=1.35, 7.7, 9.1 Hz, H-6), 8.22-8.24 (1H, dd, *J*=1.2, 7.95 Hz, H-8); MS (70 eV): m/z 300 (14.43%) [M^+], 299 (64.51), 183 (3.56), 159 (58.39), 141 (1.46), 145 (1.92), 117 (100) [$C_{20}H_{28}O_2$: Calcd 300.2089; Found: 300.2104 (MS)].

2-(2-Oxotridecyl)benzoic acid 3b. A suspension of **2b** (2.4 g, 8.0 mmole in ethanol (135 mL) and potassium hydroxide (5%, 270 mL) was refluxed for 4 hr. After cooling, the reaction mixture was stripped off ethanol under reduced pressure, cold water (60 mL) added and the mixture acidified with dil. hydrochloric acid. The reaction mixture was then extracted with dichloromethane (2×40 mL), dried (Na_2SO_4) and the solvent rotatory evaporated to give a yellow oil which solidified on standing. The crude solid was recrystalized from ethyl acetate and pet. ether (60-80°) to afford **3b** (1.9 g, 6.0 mmole, 74.7%), m.p. 40°; IR (KBr): 1720, 1685, 1610 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.87 (3H, t, *J*=6.53 Hz, H-13′), 1.1-1.3 (16H, m, H-5′-12′), 1.59-1.66 (2H, p, *J*=7.44, 14.52 Hz, H-4′), 2.31-2.35 (2H, t, *J*=7.48 Hz, H-3′), 4.03 (2H, s, H-1′), 7.17-7.19 (1H, dd, *J*=1.22, 7.56 Hz, H-3), 7.34-7.37 (1H, ddd, *J*=1.19, 8.0, 9.27 Hz, H-5), 7.46-7.52 (1H, ddd, *J*=1.22, 7.58, 8.8 Hz, H-4), 8.09-8.12 (1H, dd, *J*=1.2, 7.8 Hz, H-6), 11.35 (1H, s, D_2O exchanged, COOH); MS (70 eV): m/z 318 (11.5%) [M^+], 300 (29.5) [M^+-H_2O), 191 (23.5), 163 (3.4) [$C_{20}H_{30}O_3$: Calcd 318.2195, Found 318.2145 (MS)].

3-Undecylisocoumarin 2b. Method A. Compound **3b** (200 mg, 0.63 mmole) was refluxed with acetic anhydride (2 mL) for 12 hr. After cooling, the reaction mixture was poured into ice-water and extracted with ethyl acetate (2×20 mL). The extracts were combined and washed with aqueous sodium bicarbonate (2×10 mL, 5%), water (15 mL), dried (Na_2SO_4) and concentrated to give **2b** (160 mg, 0.53 mmole, 84.6%) as a semi-solid; R_f value, mass, high resolution mass, IR and ^1H NMR spectral data were in good agreement with those of **2b** synthesized above.

Method B. A solution of **3b** (100 mg, 0.31 mmole) in dry methanol (75 mL) and conc. sulfuric acid (as a catalyst) was refluxed for 8 hr. The reaction mixture was neutralized with solid sodium bicarbonate, filtered and methanol rotatory evaporated to afford **2b** (80 mg, 0.27 mmol, 92%) as a semi-solid.

Methyl 2-(2-oxotridecyl)benzoate 4b. Method A. Compound **3b** (300 mg, 0.94 mmole), methyl iodide in excess and anhydrous potassium carbonate (2.0 g) in dry acetone (20 mL) were heated under reflux for 2 hr. The reaction mixture was filtered while hot. The cake was washed with warm dry acetone (10 mL) and the solvent evaporated *in vacuo* leaving **4b** as an oil (280 mg, 0.84 mol, 89%); R_f value, mass, high resolution mass, IR and ^1H NMR spectral data were in good agreement with those of **4b** synthesized by Method B.

Method B. A solution of **3b** (150 mg, 0.47 mmole) in dry methanol (100 mL) and conc. sulfuric acid (two drops) was refluxed for 8 hr. Water (50 mL) was then added, methanol removed under reduced pressure, and the reaction mixture extracted with ether (2×20 mL). The extracts were combined,

dried (Na_2SO_4) and the solvent was removed under reduced pressure. The compound was purified by preparative layer chromatography to give **4b** as a yellow oil (109 mg, 0.33 mmole, 70%); IR (neat): 1720, 1685 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.85 (3H, t, *J*=6.49 Hz, H-13′), 1.23-1.36 (16H, m, H-5′-12′), 1.54-1.62 (2H, p, *J*=7.17, 14.59 Hz, H-4′), 2.25-2.30 (2H, t, *J*=7.37 Hz, H-3′), 3.82 (3H, s, OMe), 4.06 (2H, s, H-1′), 7.13-7.16 (1H, dd, *J*=0.89, 7.56 Hz, H-3), 7.28-7.33 (1H, ddd, *J*=1.38, 7.66, 9.06 Hz, H-5), 7.41-7.47 (1H, ddd, *J*=1.50, 7.49, 9.00 Hz, H-4), 7.96-7.99 (1H, dd, *J*=1.27, 7.79 Hz, H-6); MS (70 eV): m/z 332 (0.92%) [M^+], 301 (2.52), 300 (100), 192 (31.25), 183 (38.65), 149 (6.58), 118 (16.29), 59 (6.13) [$C_{21}H_{32}O_3$: Calcd 332.2351; Found 332.2360 (MS)].

(*dl*)-3, 4-Dihydro-3-undecylisocoumarin 6b. Compound **3b** (1.0 g, 3.0 mmole) was heated under reflux with sodium borohydride (1.0 g) in absolute ethanol (75 mL) for 4 hr. Ethanol was rotatory evaporated, cold water (225 mL) added, and the reaction mixture acidified with dil. sulfuric acid and extracted with ethyl acetate (2×50 mL). The extract was dried over (Na_2SO_4) and solvent evaporated to leave **5a** as yellow oil (0.8 g). The crude oil was dissolved in acetic anhydride (5 mL) and heated under reflux for 2 hr. The reaction mixture was cooled, water (50 mL) added, stirred and extracted with dichloromethane (2×30 mL). The extracts were washed with sodium bicarbonate (2×20 mL, 5%), then with water (20 mL), dried (Na_2SO_4) and rotatory evaporated to leave **6b** as an oil (0.7 g, 2.3 mmole, 77%); IR (neat): 1720, 1620 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.87 (3H, t, *J*=6.9 Hz, H-11′), 1.22-1.33 (16H, m, H-3′-10′), 1.58-1.64 (2H, p, *J*=7.5, 14.7 Hz, H-2′), 1.66-1.73 (1H, m, H-1′), 1.83-1.90 (1H, m, H-1′), 2.86-2.87 (*AB* pattern, 1H, d, d, J_{vic}=3.45, J_{gem}=16.25 Hz, H-4), 2.93-2.98 (*AB* pattern, 1H, 1.4, 7.6 Hz, H-5), 7.35-7.38 (1H, ddd, *J*=1.46, 7.6, 9.0 Hz, H-7), 7.49-7.52 (1H, ddd, *J*=1.4, 7.55, 8.90 Hz, H-6), 8.07-8.08 (1H, dd, *J*=1.2, 7.75 Hz, H-8); MS (70 eV): m/z 302 (11%) [M^+], 301 (48.56), 156 (3.0), 146 (96.0), 117 (100), 90 (34.0) [$C_{20}H_{30}O_2$: Calcd 302.2246; Found 302.2236 (MS)].

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Indian Journal of Chemistry
Vol. 37B, April 1998, pp.370 - 375

Synthetic and biological studies on 5-(*p*-chlorophenyl)furan-2-carboxyl peptides and 4-[2′-(5′-formyl)furyl]benzoyl peptides

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Received 21 March 1997; accepted (revised) 5 March 1998

Substituted anilines are coupled with furoic acid and furfural at the position-5 to get 5-(*p*-chlorophenyl)furan-2-carboxylic acid and 4-[2′-(5′-formyl) furyl]benzoic acid which on further coupling with amino acid esters, di-, tetra- and hexapeptides yield 5-(*p*-chlorophenyl)furan-2-carboxylamino acid esters and peptides and 4-[2′-(5′-formyl) furyl]benzoyl amino acid esters and peptides. The structures of these compounds have been confirmed by elemental and spectral analysis. The biological activity of these compounds are reported.

Prompted by the varied biological properties of peptides[1], substituted phenyl furan derivatives[2], bromo furoyl amino acids[3], nitro furan acryloyl amino acid methyl esters[3], 2-furylacryloylamino acids and dipeptide derivatives[4], substituted furan amino acid derivatives[5] and furan peptides[6], it was contemplated to synthesise first aryl substituted furoic acid and furyl substituted benzoic acid and then condense them with different amino acid esters and peptides using DCC as the coupling agent.

Diazotised solution of *p*-aminobenzoic acid with furfural gives 4- [2′- (5′-formyl)furyl]benzoic acid **1** (cf. Scheme I). *p*-chloroaniline was substituted to furoic acid at position-5 after diazotisation to give 5-(p-chlorophenyl)furan-2-carboxylic acid **2** (cf. Scheme II),which was confirmed by undepressed mixed melting point with authentic samples[7].

For the amino group protection of L-proline, L-phenylalanine and L-valine, di-*tert*-butyl pyrocarbonate (Boc-O-Boc) was used and carboxylic group of L-proline, L-valine and L-leucine was protected by converting it in to methyl ester.

Dipeptides *N*-^{t}Boc-Pro-Pro-OMe, *N*-^{t}Boc-Pro-Leu-OMe, *N*-^{t}Boc-Pro-Val-OMe, *N*-^{t}Boc-Val-Val-OMe, *N*-^{t}Boc-Phe-Tyr-OMe were prepared from the corresponding amino acid methyl esters and ^{t}Boc-amino acids using DCC as a coupling agent and NMM as a base in dichloromethane. The reaction mixtures were stirred for 24 hr at room temperature.

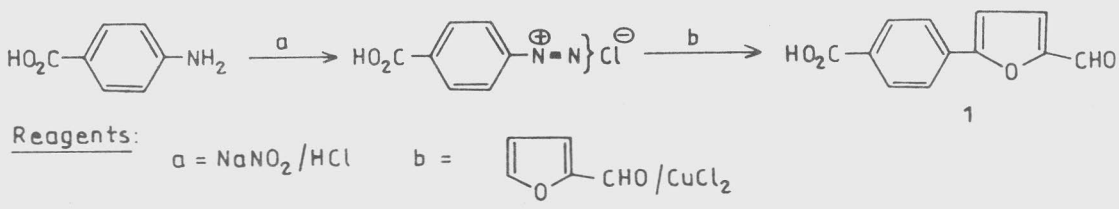
The *N*-^{t}Boc group was removed using $CF_3COOH/CHCl_3$ and the methyl ester group was removed using LiOH at room temperature.

The tetrapeptide *N*-^{t}Boc-Val-Val-Pro-Val-OMe was prepared from dipeptides *N*-^{t}Boc-Val-Val-OMe and *N*-^{t}Boc-Pro-Val-OMe using DCC and NMM after proper deprotection. Similarly, tetrapeptide *N*-^{t}Boc-Val-Pro-Phe-Tyr-OMe was prepared from dipeptides *N*-^{t}Boc-Val-Pro-OMe and *N*-^{t}Boc-Phe-Tyr-OMe after proper deprotection.

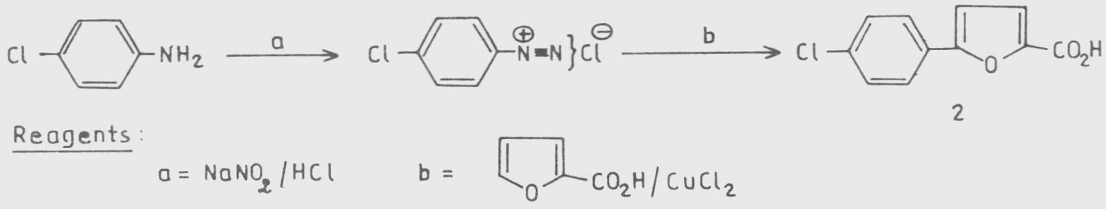
The hexapeptide *N*-^{t}Boc-Pro-Pro-Val-Pro-Phe-Tyr-OMe was prepared from *N*-^{t}Boc-Pro-Pro-OMe and *N*-^{t}Boc-Val-Pro-Phe-Tyr-OMe after proper deprotection of the amino and carboxyl groups by using DCC and NMM as the coupling reagents.

4-[2'-(5'-Formyl)furyl]benzoic acid and valine methyl ester hydrochloride were coupled using DCC and NMM. The procedure was the same as that used for the preparation of dipeptides. The resulting compound was then subjected to deprotection of the methyl ester group using LiOH to obtain 4-[2'-(5'-formyl)furyl]benzoyl valine **3a** (cf. Scheme III).

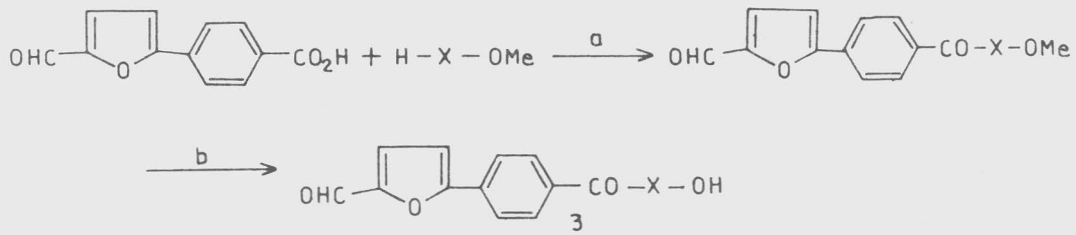
The *N*-^{t}Boc group of *N*-^{t}Boc-Pro-Leu-OMe and *N*-^{t}Boc-Pro-Pro-OMe was removed using $CF_3COOH/ CHCl_3$. The free carbonyl end of 5′-[formyl)furyl]benzoic acid and the free end of dipeptides H-Pro-Leu-OMe and H-Pro-Pro-OMe



Scheme I



Scheme II



Scheme III

were coupled to obtain 4-[2′-(5′-formyl)furyl]-benzoyl-prolyl- leucine methyl ester **3b** and 4-[2′-(5′-formyl)]benzoyl-furyl-prolyl-proline methyl ester. The methyl group of the latter compound was deprotected using LiOH (cf. Scheme III) to obtain 4-[2′-(5′-formyl)furyl]benzoyl-prolyl-proline **3c**.

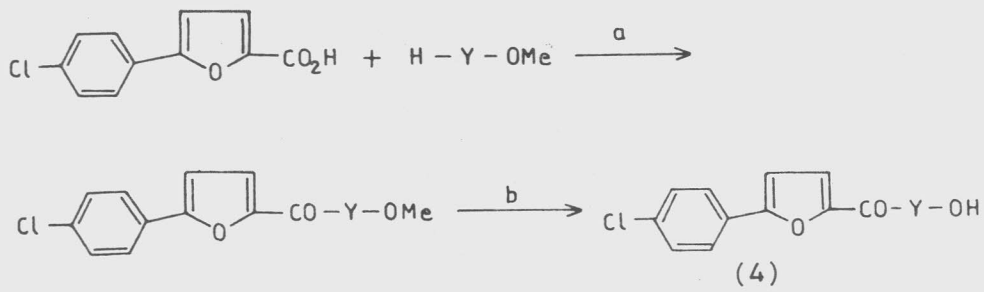
The ^{t}Boc-group of *N*-^{t}Boc-Val-Val-Pro-Val-OMe was removed using $CF_3COOH/CHCl_3$ and was coupled with 4-[2′-(5′-formyl)furyl]benzoic acid and 5-(*p*-chlorophenyl)furan-2-carboxylic acid respectively. The resulting compounds were subjected to methyl ester group deprotection to obtain 4-[2′-(5′-formyl]furyl-benzoyl-valyl-valyl-prolyl-valine **3d** and 5-(*p*-chlorophenyl)furan-2-carboxyl-valyl-valyl- prolyl- valine **4a**(cf. Scheme IV).

Similarly ^{t}Boc deprotected hexapeptide *N*-^{t}Boc-Pro-Pro-Val-Pro-Phe-Try-OMe was coupled with 5-(*p*-chlorophenyl)furan-2-carboxylic acid. The ester group of the resulting compound was removed using LiOH to obtain 5-(*p*-chorophenyl)furan-2-carboxyl-prolyl- prolyl-valyl-prolyl-phenylalanyl-tryptophan **4b** (cf. Scheme IV).

All the newly synthesized compounds were confirmed by elemental analyses and spectral data. Yieldwise, DCC is found to be the good coupling agent.

Biological activity

The antifungal and antibacterial activity of all newly synthesized compounds were tested against *Bacillus subtillis, Staphylococcus aureus, Escherichia coli, Pseudomonas aerugirosa* and



Where, Y = L-Val-L-Val-L-Pro-L-Pro,
L-Pro-L-Pro-L-Val-L-Pro-L-Phe-L-Trp

Reagents :
a = CH_2Cl_2, DCC, NMM, 24 hrs, RT
b = THF : H_2O (1:1), LiOH, 1hr, RT

Scheme IV

Table I—Antibacterial and antifungal activity of compounds **1,3a-d** and **4a,b**
Zone of inhibition (in mm) at 10μg / concentration

Compd	*B.subtillis*	*S.aureus*	*E.coli*	*P.aerugirosa*	*C.albicans*
1	—	09	09	—	—
3a	—	—	07	08	—
3b	—	08	—	0	09
3c	—	—	—	08	09
3d	—	—	09	10	—
4a	08	—	—	09	—
4b	—	09	08	—	—

Candida albicans at 10μg/mL in the neutral agar media following the disc diffusion method. Solvent control was also run to know the activity of blank solvent. The results are recorded in Table I. It was observed that the antifungal and antibacterial activities of furan derivatives were changed after coupling with amino acid esters and peptides.

Experimental Section

All the melting points were taken in open capillaries and are uncorrected. IR spectra were recorded on a Perkin-Elmer 137 instrument (λ_{max} in cm^{-1}) and ^1H NMR in continuous wave mode on an EM-390 (90 MHz) spectrometer (chemical shifts in δ,ppm) using $CDCl_3$ as a solvent and TMS as an internal standard. Purity of all compounds were checked by TLC on silica gel G plates.

Preparation of dipeptides[8]

Amino acid ester hydrochloride (10 m moles) was dissolved in $CHCl_3$ (30 mL). To this solution *N*-methyl morpholine (35 m moles) was added at 0°C. The reaction mixture was stirred for 15 minutes. Boc-amino acid (10 m moles) in CH_2Cl_2 (15 mL) and DCC (10 m moles) were added with stirring. After 24 hr, the reaction mixture was filtered, concentrated under reduced pressure and diluted with ethyl acetate. The organic layer was washed with HCl (10%), $NaHCO_3$ (10%) and saturated NaCl solutions. The organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated in vacuum. It was purified by recrystallization from ethyl acetate / *n*-hexane.

***N-tert*-Butyloxycarbonyl-prolyl-proline methyl ester 1':** Dense liquid, yield 60%, IR($CHCl_3$): 3415(br.s), 3250(br.s), 3910(s), 2960(s), 2920(s), 2840(m), 1730(m), 1695 (s), 1645(br. s), 1500(br. s), 1380(m), 1360(s), 1160(s), 1085(m), 1010(s) cm^{-1}, ^1H NMR ($CDCl_3$, 90 MHz): δ 4.5 (2H, m), 3.72(3H, s), 3.5 (4H, m), 2.3-1.9 (8H, m), 1.6-1.5 (9H, s).

***N-tert*-Butyloxycarbonyl-prolyl-leucine methyl ester 2':** Pale white crystals, m.p. 93°C, yield 66%, IR($CHCl_3$): 3250(br.s), 2950(s), 1710(s), 1700(s), 1620(s), 1510(m), 1420(m), 1380(s), 1360 (s), 1160 (s), 1020 (s), 1000(m) cm^{-1}, ^1H NMR ($CDCl_3$, 90 MHz): δ 4.2 (1H, δ, *J*=6.0 Hz), 3.7 (3H,s), 2.0-1.9 (2H,m), 1.65 (2H, t, *J*=6.5 Hz), 1.45 (9H,s), 1.35 (1H,m), 1.3-1.1 (6H,m), 1.0-0.8 (6H, d, *J*=6.5 Hz).

***N-tert*-Butyloxycarbonyl-prolyl-valine methyl ester 3':** White crystals, m.p. 40°C, yield 60%, IR($CHCl_3$): 3540(br.s), 3310(br.s), 2960(s), 2920(m), 2880(s), 1735(s), 1670(s), 1520(br. s), 1390(s), 1245(m), 1155(s), 1080(s), 1020(s), 770(s) cm^{-1}; ^1H NMR ($CDCl_3$, 90 MHz): δ 4.3 (1H, m), 3.75 (3H, s), 3.5 (1H, m), 3.4 (1H,m), 2.4-1.7 (6H,m), 1.5 (9H, s), 1.5-1.3 (1H, m), 0.9 (6H, d, *J*=6.0 Hz).

***N-tert*-Butyloxycarbonyl-phenylalanyl-tryptophan methyl ester 4':**White crystals, m.p. 144°C, yield 62%, IR($CHCl_3$): 3500(br.s), 3400(s), 3300(br.s), 2900(s), 1750(s), 1680(m), 1610(s), 1510(m), 1360 (s), 1300(s), 1220(s), 1160(m), 1010(s), 720(s), 700(s) cm^{-1}; ^1H NMR ($CDCl_3$, 90 MHz): δ 7.25-7.0 (10H, m), 6.85 (1H, br. s), 6.4(1H, br.s), 4.85(1H,m), 4.2(1H,m), 3.6 (3H, s), 3.2(2H, d, *J*=6.0 Hz), 3.0(2H, d, *J*=6.0 Hz), 1.4(9H,s).

***N-tert*-Butyloxycarbonyl-valyl-valine methyl ester 5':** White crystals, m.p. 140°C, yield 63%, IR($CHCl_3$): 3520(br.s), 3300(br.s), 2960(s), 2890(s), 1740(s), 1680(s), 1630(s), 1540(s), 1520(s), 1450(s), 1380(s), 1310(s), 1240(s), 1120(s), 1100(s), 1020(s) cm^{-1}; ^1H NMR ($CDCl_3$, 90 MHz): δ 6.5(1H,br.s), 4.6-4.4 (1H, m), 4.2-4.0 (1H, m), 3.7(3H,s), 2.7 (1H, br. s), 1.4 (9H, s), 1.4-1.0 (2H, m), 0.95(12H, d, *J*=7.5 Hz).

Preparation of tetrapeptides

N-^{t}Boc-Peptide acid (10 m moles), peptide methyl ester (10 m moles) were dissolved in $CHCl_3$ or THF. To this solution NMM (35 m moles) was added at 0°C. The reaction mixture was stirred for 15 minutes. DCC (10 m moles) was then added and stirred for 24 hr. Further work up was done as described for the preparation of dipeptides.

***N-tert*-Butyloxycarbonyl-valyl- prolyl-phenylalanyl-tryptophan methyl ester 6':** Dense liquid, yield 61%, IR ($CHCl_3$): 3300(br.s), 3020(s), 2950(br.s), 2900(s), 1730(s), 1640(m), 1510(br.s), 1430(s), 1350(s), 1240(s), 1160(s), 1090(s), 1030(s), 1000(s), 740(s) cm^{-1}, ^1H NMR ($CDCl_3$, 90 MHz): δ 7.4-6.9 (10H, m), 6.85 (1H, br. s), 6.6 (1H, br. s), 6.5 (1H, br. s), 4.9 (1H, m), 4.7 (1H, m), 4.3 (1H, m), 3.9 (1H, m), 3.65 (3H, s), 3.2 (2H, d, *J*=6.0 Hz), 3.0(2H, d, *J*=6.0 Hz), 2.2-1.6 (6H, m), 1.45 (9H, s), 1.35-1.2 (1H, m), 0.9 (6H, d, *J*=6.0 Hz), ^{13}C NMR ($CDCl_3$ 100.4 MHz): δ 218(s), 171.6(s), 171.5(s), 170.0(s), 150.8(s), 136.1(s), 136.0(s), 128.6(s), 128.5(s), 127.3(s), 126.9(d), 123.3(d), 122.0(d), 119.5(d), 118.3(d), 114.4(d), 111.3(d), 109.3(d), 79.6(d), 60.0(d), 52.8(d), 52.3(d), 49.3(d), 37.6(d), 33.9(t), 31.3(t), 30.4(t), 28.3(t), 27.5(t), 25.6(q), 24.9(q), 19.5(q), 19.0(q), 17.3(q), 16.9(q).

***N-tert*-Butyloxycarbonyl-valyl-valyl-prolyl-valine methyl ester 7':** Dense liquid, yield 60%, IR ($CHCl_3$): 3520(br.s), 3400(br.s), 3310(br.s), 2990(s), 2950(s), 2920(s), 2840(s), 1730(s), 1695(s), 1650(s), 1610(s), 1570(s), 1400(s), 1370(s), 1150(s), 1020(s), 810(s) cm^{-1}, ^1H NMR ($CDCl_3$,90 MHz): δ 7.15(1H, br.s), 6.8 (2H, br. s), 4.7-4.4 (3H, m), 4.2-4.0 (1H, m), 3.7 (3H, s), 3.6-3.3(2H,m), 2.35-1.8 (4H, m), 1.45 (9H, s), 1.35-1.1 (3H, m), 0.95 (18H, d, *J*= 6.0 Hz).

Preparation of aryl substituted furoic acid and aryl substituted furfurals[9]: A mixture of substituted aniline (100 m moles), dil. HCl (15%, 60 mL) and water (90 mL) was heated to get a clear solution. The solution was cooled to 0°C and diazotised with $NaNO_2$ solution (30%, 24 mL). The diazonium salt solution was filtered and to the filtrate, water (50 mL) and furoic acid (100 m moles) / freshly distilled furfural (100 m moles) and aqueous cupric chloride (2.5 g in 10 mL of water) were added with stirring. The stirring was continued for 4 hr and kept overnight. The separated solid was collected by filtration and washed with cold ethanol. The crude compound was recrystallized from a mixture of ethanol/DMF to obtain pure aryl substituted furoic acid /aryl substituted furfural.

4-[2'-(5'-Formyl)furyl] benzoic acid 1: Orange crystalline solid, m.p. 282°C, yield 85% (Found: C, 66.5; H, 3.7. $C_{12}H_8O_4$ requires C, 66.7; H, 3.7%); IR($CHCl_3$): 3706(s), 2820(s), 1676(s), 1660(s), 1580(s), 1510(s), 1450(s), 1400(s), 1200(s), 1050(s), 1000(s) cm^{-1}, ^1H NMR ($CDCl_3$,

90 MHz): δ 9.7 (1H, s), 8.3 (2H, d, *J*= 7.5 Hz), 7.95 (2H, d, *J*=7.5 Hz), 7.35 (1H, d, *J*=5.5 Hz), 7.0 (1H, d, *J*=5.5 Hz),

5-(4′-Chlorophenyl) furan-2-carboxylic acid 2: White solid, m.p.197°C, yield 62%, IR ($CHCl_3$): 2855(s), 1680(s), 1662(s), 1580(s), 1476(s), 1416(s), 1260(s), 1094(s), 968(s), 831(s), 792(s) cm^{-1}, H NMR ($CDCl_3$, 90 MHz): δ 9.65(1H,s), 7.75(2H,d, *J*=7.5 Hz), 7.4(2H,d, *J*=9.0 Hz), 7.3(1H,d, *J*=5.5 Hz), 6.8(1H,d, *J*=5.5 Hz).

***N-tert*-Butyloxycarbonyl-prolyl-prolyl-valyl-prolyl-phenylalanyl-tryptophan methyl ester 8′:** Dense liquid, yield 50%, IR($CHCl_3$): 3460(br.s), 3380(br.s), 3190(br.s), 2950(s), 2900(s), 1730(s), 1650(m), 1500(m), 1440(s), 1385(s), 1355(s), 1315(s), 1265(s), 1235(s), 1155(s), 1110(m), 1080(m), 920(m), 880(m), 855(m), 760(br.s) cm^{-1}, ^1H NMR ($CDCl_3$, 90 MHz): δ 7.4-6.9 (10H, m), 6.8-6.2 (4H, m), 4.9-4.0(6H,m), 3.6-3.4 (4H, m), 3.2 (2H, d, *J*=6.5 Hz), 3.0 (2H, d, *J*=7.0 Hz), 2.2-1.5 (18H, m), 1.4 (9H, s), 1.3-1.0 (1H, m), 0.9(6H,d, *J*=7.5 Hz), FABMS: m/z 856(M+1, 3%), 831(5), 781(10), 684 (35), 562(80), 306 (62), 219(58), 201(68), 120(100), 70(82).

4-[2′-(5′-formyl) furyl] benzoyl-valine 3a: Semisolid, yield 80%, (Found: C, 64.6; H, 5.4; N, 0.3. $C_{17}H_{17}O_5N$ requires C, 64.8; H, 5.5; N, 0.3%), IR($CHCl_3$): 3100(br.s), 2900(s), 2820(s), 1720 (br. s), 1660(br.s), 1570(s), 1360(s), 800(s) cm^{-1}, ^1H NMR ($CDCl_3$, 90 MHz): δ 9.7 (1H, s), 7.9 (2H, d, *J*=7.5 Hz), 7.6 (2H, d, *J*=7.5 Hz), 7.35(1H, d, *J*=4.0 Hz), 6.9 (1H, d, *J*=4.0Hz), 6.3 (1H, br. s), 4.2 (1H, m), 1.5-1.3 (1H, m), 0.96 (6H, d, *J*=6.5 Hz).

4-[2′-(5′-formyl) furyl]benzoyl-prolyl-leucine methyl ester 3b: Semisolid, yield 78%, (Found: C, 65.3; H, 6.3; N, 6.3. $C_{24}H_{28}O_6N_2$ requires C, 65.5; H, 6.4; N, 6.4%), IR ($CHCl_3$): 3360 (br.s), 3000(s), 2960(s), 1760(s), 1720(s), 1680(s), 1560(s), 1480(s), 1400(s), 1280(s), 1080(s) cm^{-1}, ^1H NMR ($CDCl_3$, 90 MHz): δ 9.7 (1H, s), 7.9 (2H, d, *J*= 7.5 Hz), 7.6 (2H, d, *J*=7.5 Hz), 7.35 (1H, d, *J*=4.0Hz), 6.9 (1H, d, *J*=4.0 Hz), 6.4 (1H, br. s), 4.5 (1H, m), 4.25 (1H, m), 3.7 (3H, s), 3.5-3.1(2H, br. m), 2.32-1.5(6H, m), 1.3-1.1 (1H, m), 0.95 (6H, d, *J*=6.0Hz).

4-[2′-(5′-Formyl)furyl]benzoyl- prolyl-proline 3c: Semisolid, yield 76%, (Found: C, 69.9; H, 5.3; N, 7.0.; $C_{22}H_{22}O_6N_2$ requires C, 69.0; H, 5.4; N, 7.1%), IR($CHCl_3$): 3080(s), 2890(s), 2800(s), 1670(s), 1650(s), 1580(s), 1500(s), 1450(s), 1350(s), 1320(s), 1220(s), 830(s) cm^{-1}, ^1H NMR ($CDCl_3$, 90 MHz): δ 9.7(1H,s) 7.9 (2H, d, *J*=7.5 Hz), 7.6 (2H, d, *J*=7.5 Hz), 7.35 (1H, d, *J*=4.0 Hz), 6.9 (1H, d, *J*=4.0 Hz), 4.2 (2H, m), 3.6-3.1 (4H, m), 2.2-1.6 (8H, m).

4-[2′-(5′-Formyl)furyl]benzoyl- valyl-valyl-prolyl-valine 3d: Semisolid, yield 82%, (Found: C, 62.1; H, 6.7; N, 9.3. $C_{31}H_{42}O_8N_4$ requires C, 62.2; H, 7.0; N, 9.4%), IR ($CHCl_3$): 3360(br.s), 3000(s), 2880(s), 1720(s), 1700(br.s), 1680(s), 1580(s), 1560(s), 1480(s), 1400(s), 1360(s), 1240(s), 780(s) cm^{-1}, ^1H NMR($CDCl_3$, 90 MHz): δ 9.7 (1H, s), 7.9 (2H, d, *J*=7.5 Hz), 7.6 (2H, d, *J*=7.5 Hz), 7.35 (1H, d, *J*=4.0 Hz), 6.9 (1H, d, *J*=4.0 Hz), 6.4-6.2 (3H, br. s), 4.3-3.9 (4H, m), 3.6-3.2 (2H, m), 2.1-1.6 (4H, m), 1.3-1.1 (3H, m), 0.95 (18H, d, *J*=6.0 Hz).

5 - (4′-Chlorophenyl)furan-2-carboxyl-valyl-valyl-prolyl-valine 4a: Semisolid, yield 82%, (Found: C,60.2; H, 6.7; N,9.0. $C_{31}H_{41}O_7N_4Cl$ requirers C, 60.3; H, 6.6; N, 9.1%), IR ($CHCl_3$): 3360(br.s), 3000(s), 2880(s), 1720(s), 1700(s), 1680(s), 1580(s), 1560(s), 1480(s), 1400(s), 1360(s), 1240(s), 780(s) cm^{-1}, ^1H NMR($CDCl_3$, 90 MHz): δ 8.3 (2H,d, *J*=7.5 Hz), 7.7 (2H, d, *J*=7.5 Hz), 7.2(1H, d, *J*=5.0 Hz), 7.0(1H, d, *J*=5.0 Hz), 6.4-6.2(3H, br.s), 4.3-3.9(4H,m), 3.6-3.2(2H, m), 2.1-1.6(4H,m), 1.3-1.1(3H,m), 0.95(18H,d,*J*=6.0 Hz).

5 - (4′-Chlorophenyl)furan-2-carboxyl-prolyl-prolyl-valyl-prolyl-phenylalanyl-tryptophan 4b: Semisolid, yield 78%, (Found: C, 64.4; H, 5.9; N, 10.2. $C_{51}H_{17}O_9N_7Cl$ requires C, 64.6; H, 6.0; N, 10.4%), IR ($CHCl_3$): 3360(br.s), 3300(br.s), 3120(br.s), 2900(s), 2850(s), 1750(br.s), 1650(br.s), 1620(s), 1560(s), 1450(s), 1360(s), 1310(s), 1240(s), 1100(s), 880(s) cm^{-1}, ^1H NMR ($CDCl_3$, 90 MHz): δ 8.3(2H, d, *J*=7.5 Hz), 8.1-7.9(5H, m), 7.7 (2H, d, *J*=7.5 Hz), 7.6-7.4 (5H, m), 7.2 (1H, d, *J*=5.0 Hz), 7.0 (1H, d, *J*=5.0Hz), 6.9 (2H, br. s), 6.1 (2H, br. s), 4.6 (3H, m), 4.0 (3H, m), 3.4-3.2 (4H, m), 3.2-2.9 (6H, m), 2.2-1.6 (12H, m), 1.4-1.2 (1H, m), 0.95 (6H, d, *J*=6.0 Hz).

Acknowledgement

The authors are thankful to Prof. Ramana, Department of Pharmaceutics, NGSM College of Pharmacy, Deralakatte for the antifungal and

antibacterial screening of the newly synthesized compounds.

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Indian Journal of Chemistry
Vol. 37B, April 1998, pp.376 - 381

Synthesis and antiinflammatory activity of some 3-(2-thiazolyl)-1,2-benzisothiazoles

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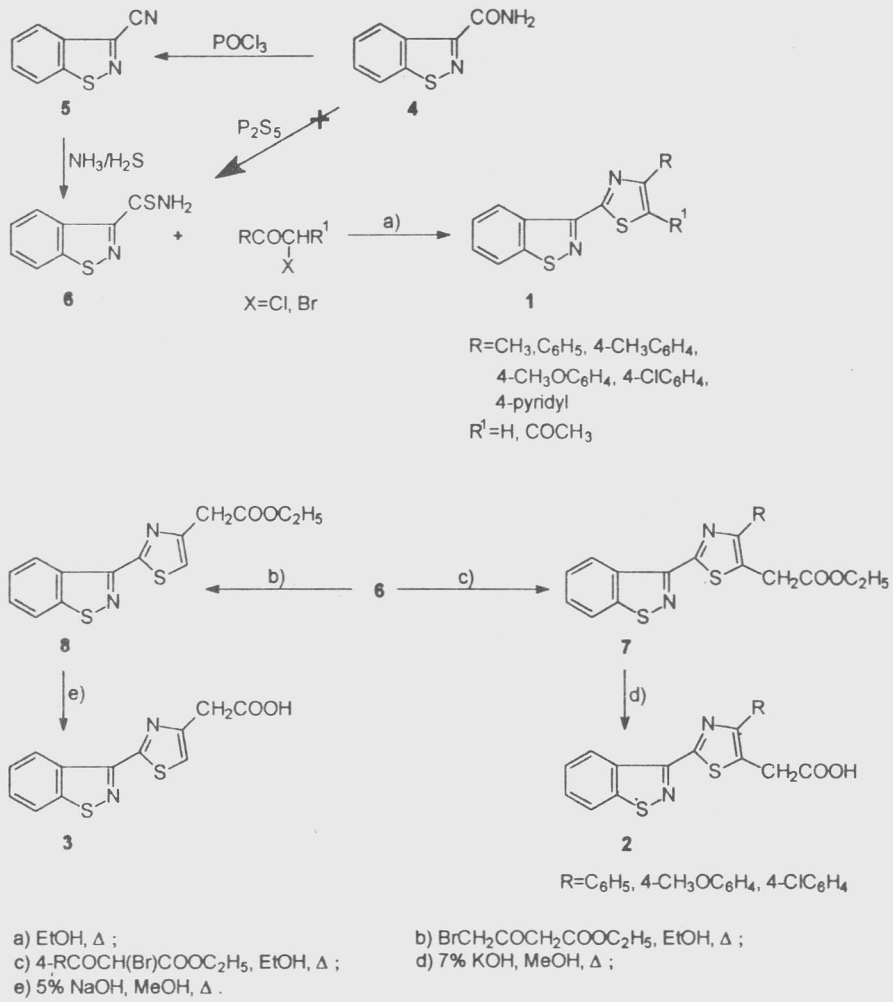
Received 17 June 1997; accepted (revised) 9 December 1997

Four types of benzisothiazole derivatives, 3-(4-alkyl/aryl-2-thiazolyl)/1,2-benzisothiazoles **1a-f** 3-(4-aryl-5-carboxymethyl-2-thiazolyl)-1,2-benzisothiazoles **2**, 3-(4-carboxymethyl-2-thiazolyl)-1,2-benzisothiazole **3** and 3-(5-acetyl-4-methyl-2-thiazolyl)-1,2-benzisothiazole **1g** have been synthesized as potential antiinflammatory agents. All the target compounds and some selected intermediates have been assayed for their antiinflammatory activity. some of these compounds possess excellent level of antiinflammatory activity.

The search for more effective non-steroidal antiinflammatory drugs (NSAIDs) has led medicinal chemists to explore a wide variety of chemical structures. A majority of these compounds, especially those with proven clinical efficacy, are acidic in nature such as aspirin, indomethacin, flufenamic acid, ibuprofen, etc. Since the discovery of aspirin, much efforts have been concentrated to the development of acedic NSAIDs and some of these, having an acetic acid grouping[1,2] are found to possess significant antiinflammatory activity. Appreciation of these findings and as a part of our ongoing programme[3,4] towards the development of NSAIDs, coupled with the observation that several 1,2-benzisothiazoles and their 1,1-dioxides possess good antiinflammatory activity[5], our attention has been directed on the variation of the 1,2-benzisothiazole moiety by introducing heterocyclic systems bearing acetic acid function with a view to synthesize new analogues with improved antiinflammatory activity[6]. In this communication, we report the synthesis of a few series of compounds such as 3-(4-alkyl or arylthiazol-2-yl)-1,2-benzisothiazoles **1a-f**, 3-(5-acetyl-4-methylthiazol-2-yl)-1,2-benzisothiazole **1g**, 3-(4-aryl-5-carboxymethyl-thiazol-2-yl)-1,2-benzisothiazoles **2** and 3-(4-carboxymethylthiazol-2-yl)-1,2-benzisothiazole **3**. These compounds have been synthesized following the reaction sequence shown in Scheme I.

The conventional method for preparing thioamides by treating the corresponding amides with phosphorus pentasulphide failed to give the hitherto unknown thioamide **6** from 1,2-benzisothiazole-3-carboxamide **4**. So, the required thiocarboxamide **6** was prepared from 1,2-benzisothiazole-3-carboxamide **4** through the 3-cyano compound **5**. The carboxamide **4** on treatment with phosphoryl chloride afforded 1,2-benzisothiazole-3-carbonitrile **5**[7] which on treatment with hydrogen sulphide and ammonia gas in ethanol gave 1,2-benzisothiazole-3-thiocarboxamide **6** in 72% yield. The structure of **6** was established by IR and mass spectra. The IR spectrum displayed peaks at 3342, 3273, 3184 (NH str.) and 1622 cm^{-1} (NH bend.). Moreover, the absorption peak due to CN in the IR spectrum of carbonitrile **5** was absent in the IR spectrum of **6**. The mass spectrum of **6** showed the molecular ion $[M]^+$ at m/z 193.9977 (calcd Mol. wt 193.9978). Condensation of **6** with chloroacetone, phenacyl bromides and 1-acetyl-1-bromoacetone according to the well known Hantzsch thiazole synthesis gave **1** in 66-81% yields (Table I).

Compound **6** on condensation with various ethyl β-aroyl-β-bromopropionates gave 3-(4-aryl-5-carbethoxymethylthiazol-2-yl)-1,2-benzisothiazoles **7** in good yields (65-68%, cf. Table I) which on alkaline hydrolysis in methanol followed by acidification afforded the corresponding acids **2** in 91-97% yields (Table I). The IR spectra of all the esters **7** showed an intense peak at 1730 cm^{-1} (C=O). The hydrolysed acids **2** exhibited apart



Scheme I

from a band at 1695 cm^{-1} (C=O), a broad band in the region 2800-2600 cm^{-1} due to hydrogen bonded OH stretching. The characteristic triplet-quartet pattern present in the ^1H NMR spectra of **7** was absent in the ^1H NMR spectra of **2**. The signal for CH_2 protons of CH_2COOH group in **2** and **7** appeared as a singlet at δ3.9.

1,2-Benzisothiazole-3-thiocarboxamide **6** also underwent condensation with ethyl γ-bromoacetoacetate smoothly in ethanolic solution giving the required 3-(4-carbethoxymethylthiazol-2-yl)-1,2-benzisothiazole **8** in 64% yield which on basic hydrolysis with 5% sodium hydroxide in methanol afforded the corresponding acetic acid **3**. The IR spectrum of the ester **8** showed a band at 1725 cm^{-1} (C=O), whereas the acid showed peaks at 1695 (C=O) and a broad peak in the region 2800-2600 cm^{-1} (hydrogen bonded OH of acid). The signal due to CH_2 protons of CH_2COOH group appeared as singlet at δ 3.8 in the ^1H NMR spectra of **3** and **8.**

Antiinflammatory activity. Compounds **1-3,6** and **7** were tested for their antiinflammatory activity by acute carrageenin-induced rat paw edema test[8]. The compounds were administered as suspension in gum acacia (1% w/v) in normal saline. Male albino Charles Foster rats weighing between 110 and 140 g were divided into groups of four each. Edema was induced by injecting 0.1 mL of carrageenin solution into the left hind paw. The compounds were administered at a dose of 100 mg/kg orally one hour before or intraperitoneally half an hour before carrageenin injection. The paw volume of the limbs was measured with a volume differential meter immediately before and 2 hr and 3.5 hr after carrageenin injection. In every set of experiments,

Table I — Characterization data of new compounds prepared

Compd[a]	R	R^1	m.p. °C	Yield (%)	Mol.formula (M^+)[b]	Calcd (%) (Found) C	H	N
1a	CH_3	H	112	63	$C_{11}H_8N_2S_2$	(56.9	3.4	12.1)
					(232.0152)	56.6	3.3	12.2
1b	C_6H_5	H	128	75	$C_{16}H_{10}N_2S_2$	(65.3	3.4	9.5)
						65.2	3.3	9.4
1c	4-$CH_3C_6H_4$	H	136	66	$C_{17}H_{12}N_2S_2$	(66.2	3.9	9.1)
					(308.0294)	66.0	3.6	9.0
1d	4-$CH_3OC_6H_4$	H	120	69	$C_{17}H_{12}N_2OS_2$	(63.0	3.7	8.6)
						62.7	3.5	8.6
1e	4-ClC_6H_4	H	153	81	$C_{16}H_9ClN_2S_2$	(58.4	2.7	8.5)
					(327.9823/ 329.9795)	58.4	2.5	8.3
1f	4-pyridyl	H	260	71	$C_{15}H_9N_3S_2$	(61.0	3.1	14.2)
						61.1	3.3	14.3
1g	CH_3	$COCH_3$	176	75	$C_{13}H_{10}N_2OS_2$	(56.9	3.6	10.2)
					(274.0176)	57.0	3.7	10.0
2a	H	–	235	94	$C_{18}H_{12}N_2O_2S_2$	(61.4	3.4	8.0)
					(352.0207)	61.7	3.6	8.1
2b	OCH_3	–	207	91	$C_{19}H_{14}N_2O_3S_2$	(59.7	3.7	7.3)
					(382.0379)	59.6	3.5	7.0
2c	Cl	–	245	97	$C_{18}H_{11}ClN_2O_2S_2$	(55.9	2.8	7.2)
						55.6	2.6	7.5
3	–	–	185	88	$C_{12}H_8N_2O_2S_2$	(52.2	2.9	10.1)
					(276.0033)	52.3	3.1	10.0
7a	H	–	128	68	$C_{20}H_{16}N_2O_2S_2$	(63.2	4.2	7.4)
					(380.0654)	63.0	4.2	7.3
7b	OCH_3	–	119	65	$C_{21}H_{18}N_2O_3S_2$	(61.5	4.4	6.8)
					(410)	61.4	4.1	6.7
7c	Cl	–	134	68	$C_{20}H_{15}ClN_2O_2S_2$	(57.9	3.6	6.8)
						57.6	3.5	6.5
8	–	–	96	64	$C_{14}H_{12}N_2O_2S_2$	(55.3	4.9	9.2)
						55.0	4.3	9.2

[a]All the compounds were crystallized from ethanol.
[b]Correct masses were found by HRMS

one group of fats was kept as control and administered only the vehicle 1% gum acacia, whereas another group received a standard drug (ibuprofen) for comparison. The results were evaluated as percent inhibition as compared with the control group. Local irritant action was tested by applying different concentrations of test compounds on the rabit cornea[9]. The results are given in Table II.

Although the compounds tested do not follow any general pattern in exhibiting AI activity, certain observations are worthmentioning. Most of the compounds show higher inhibition when administered intraperitoneally as compared to the oral route. The activity decreases rapidly with the passage of time as inhibition measured after 2 hr period is generally more than that observed after 3.5 hr indicating thereby that these compounds are rapidly metabolised in the system. Amongst the target compounds the highest activity was exhibited by compound **2c** (inhibition 75% p.o.) followed by **3** with inhibition of 64% (p.o.). Moreover, the compound **2c** had quite high activity (69% inhibition) even at dose level of 50 mg/kg. This is somewhat expected because these compounds (**2c** and **3**) belong to the family of heterocyclyalkanoic acids. The high activity puts these compounds in a class of potential NSAIDS and therefore they have been marked for detailed pharmacological screening. The order of activity shown by the compounds within the series **2** i.e. **2c>2b>2a** is in confirmation of our earlier observation that compounds with chloro or methoxy substituents show higher activity[10]. Another compound **6** has

Table II — Antiinflammatory activity of compounds **1-3,6,7** on oral (p.o.) and intraperitoneal (i.p.) administration

Compd	% inhibition			
	oral (p.o.)		intraperitoneal (i.p.)	
	2 hr	3.5 hr	2 hr	3.5 hr
1a	32	22	ND	ND
1b	7	23	12	N
1c	9	N	N	N
1d	30	7	64	30
1e	17	35	N	N
1f	6	N	43	29
1g	N	7	20	N
2a	15	19	45	7
2b	36	33	40	24
2c*	75	62	78	67
	69**	61	76	64
3	64	46	55	64
6	67	61	45	30
7a	7	11	ND	ND
7b	20	29	34	30
7c	34	22	24	13
Ibuprofen	62	65	70	74

Each value is the mean of four animals.
N≤10% inhibition
ND denotes not done.
*Value is the mean of eight animals.
**Percent inhibition at 50 mg/kg.

shown activity comparable with that of ibuprofen and has also been chosen for detailed pharmacological study.

Experimental Section

General. Melting points were determined in open capillaries in a sulphuric acid-bath and are uncorrected. IR spectra were scanned as nujol mulls on a Perkin-Elmer 842 infrared spectrophotometer (ν_{max} in cm^{-1}). ^1H NMR spectra were recorded on a Perkin-Elmer R-32 (90 MHz) instrument (chemical shifts in δ, ppm) using TMS as internal standard, and mass spectra at 70 eV on MS-12, DS-55 and MS 30/DS 50S mass spectrometers.

1,2-Benzisothiazole-3-carboxamide **4**[11], ethyl β-aroyl-β-bromopropionates[12], ethyl γ-bromoaceto-acetate[13] and 1-acetyl-1-bromoacetone[14] were prepared according to the literature methods.

1,2-Benzisothiazole-3-carbonitrile 5. 1,2-Benzisothiazole-3-carboxamide **4** (1.78 g, 0.01 mole) was placed in a round bottomed flask and phosphoryl chloride (5 mL) added to it. The reaction mixture turned hot with the dissolution of the carboxamide. The solution was then refluxed for 1 hr, cooled and poured into cold water. The resulting solid was filtered, dried under vacuum, and crystallized from ethanol, mp 91°C (Lit[7], mp 83-85°C), yield 1.3 g (81%); IR : 2234 (C-N str.).

1,2-Benzisothiazole-3-thiocarboxamide 6. 1,2-Benzisothiazole-3-carbonitrile **5** (1.6 g, 0.01 mole) was dissolved in ethanol (20 mL). Ammonia gas was passed through the solution till saturated, followed by passing of the dry hydrogen sulphide gas. The product 1,2-Benzisothiazole-3-thiocarboxamide **6** crystallized out rapidly on standing of the reaction mixture for sometime at room temperature, filtered, washed with cold ethanol, dried and crystallized from ethanol mp 98°C, yield 1.4 g (72%); IR: 3342, 3273, 3184 (NH str.), 1622 (NH bend.); ^1H NMR ($CDCl_3$): 7.2-7.6 (m, 2H, Ar-H), 7.6-7.9 (m, 1H, Ar-H), 8.3-8.8 (bs, NH), 9.1-9.4 (m, 1H, Ar-H); MS: M^+ at m/z 193.9977; Calcd Mol. wt. 193.9978. Anal. Calcd for $C_8H_6N_2S_2$: C,49.5; H, 3.1; N, 14.4. Found: C, 49.0; H, 3.3; N, 14.0%.

3-[4-(*p*-Toluyl)thiazol--2-yl]-1,2-benzisothiazole 1c. A mixture of 1,2-Benzisothiazole-3-thiocarboxamide **6** (1.94 g, 0.01 mole) and *p*-methylphenacyl bromide (2,13 g, 0.01 mole) in ethanol (50 mL) was refluxed for 6 hr. The volume was reduced to half and the solid which separated out was filtered and treated with aqueous ammonia. The product was collected by filteration, washed with water, dried and crystallized from ethanol, mp 136°C, yield 2.0 g (66%); IR: No absorption in the region 3400-3100 and 1800-1650 (NH and C=O absent); ^1H NMR ($CDCl_3$) : 2.4 (s, 3H, CH_3), 7.1-8.1 (m, 8H, Ar-H and thiazole 5-H), 9.1-9.4 (m, 1H, Ar-H); MS : M^+ at m/z 308.0294; Calcd Mol. wt. 308.0447.

Other compounds in the series were prepared similarly and are listed in Table I.

3-[4-(*p*-Anisyl)-5-carbethoxymethylthiazol-2-yl]-1,2-benzisothiazole 7b. To a solution of 1,2-benzisothiazole-3-thiocarboxamide **6** (1.94 g, 0.01 mole) in ethanol (25 mL) was added a solution of ethyl β-(*p*-anisoyl)-β-bromopropionate (2.85 g, 0.01 mole) in ethanol (25 mL). The reaction mixture was refluxed for 4 hr, cooled and kept overnight. The crystalline solid which separated out was filtered, washed with sodium bicarbonate solution (2%) and then with water, dried and crystallized from ethanol, mp 119°C, yield 2.7 g

(65%); IR : 1730 (C=O str. of $COOC_2H_5$); ^1H NMR ($CDCl_3$) : 1.25 (t, 3H, $COOCH_2CH_3$), 3.8 (s, 3H, OCH_3), 3.9 (s, 2H, $CH_2COOC_2H_5$), 4.2 (q, 2H, $COOCH_2CH_3$), 6.8-8.0 (m, 7H, Ar-H); MS : M^+ at m/z 410; Calcd mol. wt. 410.

Other compounds in the series were prepared similarly and are listed in Table I.

3-[4-(*p*-Anisyl)-5-carboxymethylthiazol-2-yl]-1,2-benzisothiazole 2b. To a solution of 3-[4-(*p*-anisyl)-5-carbethoxymethylthiazol-2-yl]-1,2-benzisothiazole **7b** (4.10 g, 0.01 mole) in methanol (20 mL) was added potassium hydroxide solution (7%, 20 mL) and the mixture heated under reflux for 1 hr. After cooling, the solution was filtered from any undissolved matter and the filtrate acidified with glacial acetic acid. The product so obtained was filtered, washed with water, dried and crystallized from ethanol, mp 207°C, yield 3.48 g (91%); IR : 2800-2600 (hydrogen bonded OH of COOH), 1694 (C=O str.); ^1H NMR (DMSO-d_6 $CDCl_3$) : 3.8 (s, 3H, OCH_3), 3.9 (s, 2H, CH_2COOH), 6.7-8.1 (m, 7H, Ar-H), 8.9-9.2 (m, 1H, Ar-H),; MS: M^+ at m/z 382.0379; Calcd Mol. wt. 382.0451.

Other compounds in the series were prepared similarly and are listed in Table I.

3-(4-carbethoxymethylthiazol-2-yl)-1,2-benzisothiazole 8. To an ice cold solution of ethyl γ-bromoactoacetate (2.09 g, 0.01 mole) in ethanol (15 mL) was added a solution of 1,2-benzisothiazole-3-thiocarboxamide **6** (1.94 g, 0.01 mole) in ethanol (20 mL) and the mixture stirred for 15 hr. The unreacted ester was removed on shaking with ether. The aqeous layer was neutralized with aqeous sodium bicarbonate. The solid which separated out was filtered, washed with sodium bicarbonate solution (2% and then with water, dried and crystallized from ethanol, mp 96°C, yield 1.95 g (64%); IR : 1725 (C=O str. of $COOC_2H_5$); ^1H NMR ($CDCl_3$): 1.3 (t, 3H, $COOCH_2CH_3$), 3.95 (s, 2H, $CH_2COOC_2H_5$), 4.2 (q, 2H, $COOCH_2CH_3$), 7.2-8.1 (m, 4H, Ar-H and thiazole 5-H), 9.0-9.3 (m, 1H, Ar-H). Anal. Calcd. for $C_{14}H_{12}N_2O_2S_2$: C, 55.3; H, 3.9; N, 9.2. Found: C, 55.0; H, 4.3; N, 9.2%.

3-(4-carboxymethylthiazol-2-yl)-1,2-benzisothiazole 3. A suspension of 3-(4-carbethoxymethylthiazol-2-yl)-1,2-benzisothiazole **8**. (1.25 g, 0.005 mole) in sodium hydroxide solution (5%, 25 mL) was refluxed for 2 hr, cooled and diluted with water. It was filtered to remove any undissolved impurity and the filtrate rendered acidic with acetic acid. The solid product, thus obtained, was filtered, washed with water, dried and crystallized from ethanol, mp 185°C, yield 1.2 g (88%); IR : 2800-2600 (hydrogen bonded O-H of COOH), 1695 (C=O str.); ^1H NMR (DMSO-d_6/$CDCl_3$) : 3.8 (s, 2H, CH_2COOH), 6.9-8.1 (m, 4H, Ar-H and thiazole 5-H), 8.8-9.2 (m, 1H, Ar-H); MS : M^+ at m/z 276.0033; Calcd. Mol. wt. 275.9758. Anal. Calcd for $C_{12}H_8N_2O_2S_2$: C, 52.2; H, 2.9; N, 10.1. Found: C, 52.3; H, 3.1; N, 10.0%.

3-(5-Acetyl-4-methylthiazol-2-yl)-1,2-benzisothiazole 1g. To a solution of 1-acetyl-1-bromoacetone in ethanol (30 mL) was added a solution of 1,2-benzisothiazole-3-thiocarboxamide **6** (1.94 g, 0.01 mole) in ethanol (15 mL) and the mixture refluxed for 3 hr. The crystalline solid which separated out on cooling was filtered and treated with aqeous ammonia. The solid product was collected by filtration, washed with water, dried and crystallized from ethanol, mp 176°C, yield 2.05 g (75%); IR : 1667 (C=O str.); ^1H NMR ($CDCl_3$): 2.6 (s, 3H, CH_3), 2.85 (s, 3H, $COCH_3$), 7.1-8.1 (m, 3H, Ar-H), 9.0-9.3 (m, 1H, Ar-H); MS : M^+ at m/z 274.0176; Calcd. Mol. wt. 274.0240. Anal. Calcd for $C_{13}H_{10}N_2OS_2$: C, 56.9; H, 3.6; N, 10.2. Found: C, 57.0; H, 3.7; N, 10.0%.

Acknowledgement

The authors are thankful to Dr R S Kapil, Director, Regional Research Laboratory, Jammu for providing facilities for biological testing and to the University Grants Commission, New Delhi for financial assistance.

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Indian Journal of Chemistry
Vol. 37B, April 1998, pp.382 - 386

A new ketosteroid from the bark of *Couropita guianensis* Aubl.

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Chemical examination of the bark of *Couropita guianensis* Aubl. furnishes a new ketosteroid, couropitone in addition to β-amyrin, βamyrone, β-amyrin acetate, stigmasterol, ergosta-4,6,8(14), 22-tetraen-3-one, β-sitosterol and its glyco-side.The structure of couropitone is established as stigmasta-4,23(*E*)-dien-3-one **1**.

Couropita guianensis Aubl. (Cannonball tree, Fam: Lecythidauae) is a native of South America and Trinidad and planted in India near temples for its beautiful flowers. The large size fruit requires more than a year to ripen and the seeds are embedded in a stinking pulp[1]. Various parts of this tree are reported to be used for the treatment of skin diseases[2,3]. Different parts of this tree have been examined earlier and reported to contain oils, phenolic substances, acids[4,5], coloured matter, anthocyanidin and flavonoid glycosides[6,7], terpenoids[8,9], alkaloids and others[10,11].

The bark of the tree available in the university campus was collecged, dried and powdered. The dry powder was repeatedly extracted with ethanol in a soxhlet apparatus. The concentrate from the combined ethanolic extract was fractionated into hexane, benzene and methanol successively. The residues obtained from the individual solvent fractions were chromatographed over silica gel columns. The eluate fractions on evaporation and crystallisation of the solids obtained therefrom gave in all eight compounds. One of these happened to be a new ketosteroid, couropitone whose structure has been established as stigmasta-4,23(*E*)-dien-3-one **1** by a study of its physical and spectroscopic data and chemical reactions. The other compounds were identified as β-amyrin, β-amyrone, β-amyrin acetate, stigmasterol, ergosta-4,6,8(14), 22-tetraen-3-one, β-sitosterol and its glycoside. Although β-amyrin was reported earlier from this species, the presence of β-amyrin acetate and β-amyrone have now been reported. Similarly the report on the presence of steroid conjugated ketones including the new derivative, couropitone is quite significant.

The new ketosteroid, named couropitone, was obtained from the hexane-benzene (3:7) column fractions of the hexane soluble material and crystallised from methanol as colourless needles, m.p. 103-04°; $[\alpha]_D$+38.18° (*c* 0.16 in $CHCl_3$). Its molecular formula was fixed as $C_{29}H_{46}O$ based on elemental analysis and molecular ion M^+ at m/z 410 in its EIMS. Its molecular formula and ^1H NMR spectrum suggested it to be a steroid derivative. The UV absorption (241 nm) indicated the presence of an α,β-unsaturated kketone moiety which was suported by its IR absorption at 1670 cm^{-1} and formation of 2,4-dinitrophenylhydrazone derivative as shining orange needles. The presence of single oxygen present in the molecule in the form of a ketone indicated 3-keto functionality in the place of ubiquitous 3β-hydroxyl. This also locates one double bond in its conjugation (Δ^1 or Δ^4) more likely at C-4 considering its UV maximum.

The fragment ions m/z 271 (M^+–side chain, 32%) and 269 (M^+–side chain–2H, 25%) suggested the presence of a ten-carbon side chain $C_{10}H_{19}$ with a double bond in it (cf. Chart 1). The ^1H NMR spectrum showed the presence of six methyls, two tertiary as singlets at δ 0.72 (18-H_3) and 1.18 (19-H_3), three secondary methyls at δ 1.05 (6H, d, *J*=7 Hz, 26-H_3 and 27-H_3), 0.84 (3H, d, *J*=7 Hz, 21-H_3) and a primary methyl at 0.89 (3H, t, *J*=7 Hz, 29-H_3) reminiscent of a stigmastane skeleton. The above methyl chemical shifts closely agree with those of 23(*E*)-stigmasta-5,23-dien-3β-ol but for the 19-H_3 which appeared at δ 1.16 as in stigmasta-4-en-3-one[14]. Two protons were noticed in the olefinic region, one at δ 5.70 as a singlet and the other at

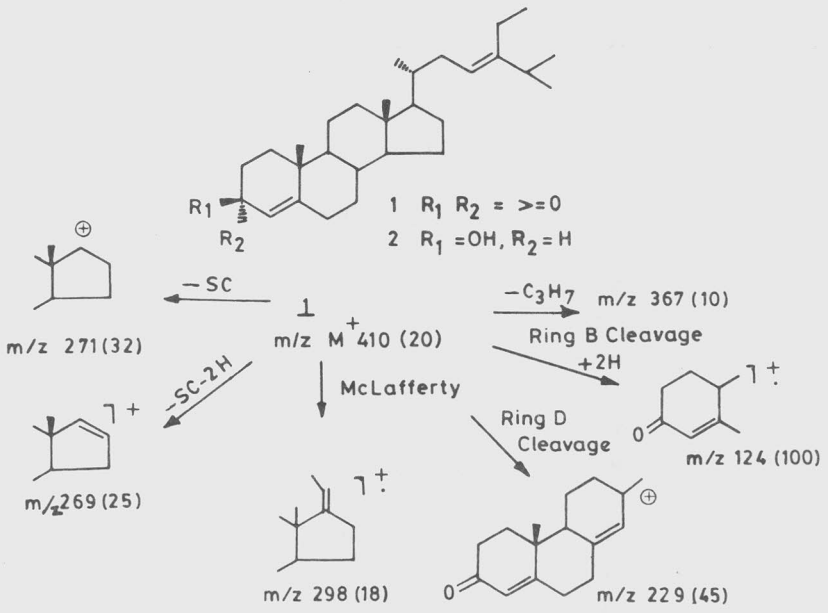

Chart 1

5.10 as a multiplet. The former could be assigned to the α-proton of an α,β-unsaturated ketone accounting for C_4-H. The second proton obviously corresponds to a trisubstituted double bond in the side chain. The absence of a methyl on double bond rules out the location of this double bond at $\Delta^{20(22)}$ or $\Delta^{24(28)}$ leaving it to be located at Δ^{23} position. The presence of 23-24 double bond was also supported by the fragment ion m/z 298 ($M^+-C_8H_{16}$) (cf. Chart 1) formed by McLafferty rearrangement with the transfer of 17-H and cleavage of 20-22 bond[13-18].

The configuration *E* or *Z* of D^{23} bond can be determined by the ^1H and ^{13}C NMR[19,20] chemical shifts of the side chain protons and carbons as assigned in cyclosadol and others[21,22]. In case of *Z*-configuration the isopropyl methane proton (25-H) appears deshielded at ≈ δ 2.2 compared to the same in *E*-isomers at ≈ δ 2.8. In couropitone the 25-H appeared mixed up in the multiplet at δ 2.3 with 28 methylene protons but no signal was found below 2.3 in support of *E*-configuration of Δ^{23}. The ^{13}C NMR spectrum of couropitone could not be obtained for want of sufficient sample. The foregoing evidence, however, fully supported the structure of couropitone as stigmasta - 4,23-(*E*)-dien-3-one **1**.

This compound has not been isolated so far from a natural source. It was however, reported as an intermediate by a series of reactions from fucosterol which is stigmasta-5,24(28)-dien-3β-ol[23]. The 23(*Z*) isomeric dienone was also reported from sargasterol[24]. Unfortunately, except for the melting points of the two dienones (110-111.5° and 121-122.5° respectively) no other physical or spectral characteristics are available. The m.p. of couropitone (103-04°) was found to be nearer to the ketone obtained from fucosterol suggesting their possible identity.

In order to provide further chemical evidence, couropitone was reduced with $NaBH_4$ in dry methanol to give an alcohol (over 80%). The alcohol **2**, $C_{29}H_{48}O$, crystallised from chloroform as colourless needles, m.p. 132-33°; $[\alpha]_D$ +64.04° and showed hydroxylic (3500 cm^{-1}) but no carbonyl absorption in its IR spectrum. It was found to be transparent in UV above 210 nm. Its ^1H NMR spectrum showed two trisubstituted olefinic protons, one at δ 5.10 as a multiplet for C_{23}-H and another at 5.30 as a doublet accounting for C_4-H of Δ^4-ene. Further, the C_3-αH was observed as a broad multiplet ($W_{1/2}$ = 20 Hz) at δ 4.21 as noticed in 3β- and 3α-hydroxy-Δ^4-cholestenes[25]. If it were a Δ^5-ene the C_3-α or β-H would have appeared around δ 3.5. Even in the alcohol **2** the isopropyl proton (25-H) appeared unaltered at δ 2.30 in support of 23(*E*)-configuration. The structure of alcohol **2**, which is again new in literature, could thus be derived as stigmasta-4,23(*E*)-dien-3β-ol.

Experimental Section

General. All melting points were determined on a VEB Analytik Dresden HMK hot plate and are uncorrected. Acme's Si gel-G was used for column and TLC. IR spectra were measured on a Shimadzu IR-408 spectrophotometer and UV spectra on a Milton Roy UV-vis spectrophotometer. ^{1}H NMR spectra were recorded on a Perkin-Elmer R-32 and Jeol-Ex 90, 90 MHz NMR spectrometers in $CDCl_3$ with TMS as internal standard (chemical shifts in δ-scale). Mass spectra were recorded on Hitachi RMU-6E and Jeol JMS-300 instruments.

Extraction, isolation and separation of compounds. The dry bark powder (2 kg) of *Couropita guianeusis* was repeatedly extracted in a soxhlet apparatus with ethanol. The extract (10 L) was concentrated to a small volume (1L) and then fractionated into *n*-hexane, benzene and methanol solubles.

Compounds fdrom *n*-hexane extract. The reddish-brown hexane extract (2 L) was concentrated to a small volume (200 mL) and left for a few days at room temperature when a colourless solid, 200 mg (β-amyrin), separated out. The solvent from the mother liquor was removed and the concentrate absorbed on Si gel (100 g) and the material subjected to column chromatograpahy over Si gel eluting with hexane and hexane-benzene mixtures collectiang faractions of 800 mL each to furnish the following compounds. The hexane fractions 1-20 and hexane-benzene (9:1) fractions 21-30 furnished an oily fatty substance (300 mg).

β-Amyrin acetate. The hexane-benzene (8:2) fractions on evaporation left a solid which on crystallisation from chloroform-methanol gave colourless shining plates (50 mg), m.p. 240-42°; $[\alpha]_D$ +84° (*c* 1.0 in $CHCl_3$), identical in its physical and spectral characteristics with β-amyrin acetate[26].

β-Amyrone. The hexane-benzene (7:3) fractions on evaporation left a solid which on crystallisation from chloroform-methanol furnished colourless needles (50 mg), m.p. 175-76°; $[\alpha]_D$ +95° of β-amyrone, identified by comparison of its physical and spectral characteristics[6] and by direct comparison with an authentic sample.

β-Amyrin. The hexane-benzene (6:4) fractions 66-85 on evaporation furnished a solid which crystallised from methanol as long colourless needles (800 mg), m.p. 199-200°; $[\alpha]_D$ +94.2° (*c* 1.54 in benzene), identified as β-amyrin by comparison of its physical and spectral characteristics and by direct comparison with an authentic sample[6,8].

Mixture of β-sitosterol and stigmasterol. The hexane-benzene (1:1) column fractions 86-100 left a solid sterol mixture (200 mg) which was acetylated with pyridine and Ac_2O (5 mL each) and the acetate obtained on work-up was separated by preparative TLC over $AgNO_3$ impregnated Si gel into two compounds. The less polar compound, eluted by chloroform-methanol, crystallized as colourless needles (100 mg), m.p. 126-27°;$[\alpha]_D$ −38.2°, and was found identical in every respect with β-sitosteryl acetate. The more polar compound, eluted by chloroform-methanol, crystallized as colourless plates (50 mg), m.p. 142-43°;$[\alpha]_D$ − 42.3°, and was identified as stigmasteryl acetate.

Ergosta-4,6,8(14),22-tetraen-3-one[27]. The hexane-benzene (4:6) fractions 101-105 furnished a solid which crystallized from methanol as colourless needles, 80 mg, m.p. 80-81°;$[\alpha]_D$ +562° (*c* 1.1 in $CHCl_3$); R_f 0.76 (benzene-EtOAc, 4:1). Anal. Calcd for $C_{28}H_{40}O$: C,85.71; H, 10.20. Found: C, 85.66; H, 10.06%; UV (MeOH): 238, 282, 348 nm; IR (KBr): 1670, 1640, 1595, 968, 873, 760, 695 cm^{-1}.

^{1}H NMR (90 MHz): 0.81, 0.90, 0.96, 1.00, 1.10 (all methyls), 5.25 (2H, m, C_{22} and C_{23}-H), 5.74 (1H, s, C_4-H), 6.03, 6.60 (1H each, d, *J*=9Hz, C_6- and C_7-H); MS: m/z 392 (M^+, 18%), 364 (4), 349 (8), 321 (4), 268 (100), 267 (80), 253 (32), 250 (18), 214 (25), 173 (30), 124 (18). A comparison of the above data with those reported for ergosta-4,6,8(14), 22-tetraen-3-one proved its identity.

Couropitone, stigmasta-4,23-diene-3-one 1. The hexane-benzene (3:7) colouman fractions 106-115 on concentration left a solid which crystallised from methanol as colourless needles (20 mg), m.p. 103-04°; $[\alpha]_D$ +38.18° (*c* 0.16 in $CHCl_3$); R_f 0.38 (benzene -EtOAc, 4:1). Anal. Calcd for $C_{29}H_{46}O$: C, 84.88; H, 11.22. Found: C, 84.82; H, 11.12%; UV (MeOH): 241 nm; IR: (KBr): 2950, 1670, 1420, 1380, 1090, 980 and 970 cm^{-1}; ^{1}H NMR (90 MHz): 0.72 9 (3H, s, 18-H_3), 0.84 (3H, d, *J*= 7Hz, 21-H_3), 1.18 (3H, s, 19-H_3), 2.30 (2H, d, *J*=7 Hz, 28-CH_2), 5.10 (1H, m, C_{23}-H), 5.70 (1H, s, C_4-H); MS: m/z 420 (M^+, 20%), 367 (10), 298 (18), 283 (8), 271

(32), 269 (25), 229 (45), 175 (25), 124 (100), 107 (50), 95 (60).

2,4-Dinitrophenylhydrazone of stigmasta-4,23-diene-3-one. To couropitone (5 mg) in methanol (5 mL) was added 2,4-dinitrophenylhydrazine (10 mg) in methanol (5 mL) and a drop of conc. HCl. The mixture was warmed for 5 min. and left overnight to give an orange solid which crystallised from methanol as shining orange needles (4 mg), m.p. 142°.

Reduction of couropitone with $NaBH_4$: Isolation of stigmasta-4,23-diene-3β-ol 2. To couropitone (10 mg) in methanol (15 mL) was added $NaBH_4$ (20 mg) and the mixture refluxed for 6 hr on a water-bath. Usual work-up gave a solid which on initial purification by passing through a small column of Si gel and subsequent crystallisation from chloroform-methanol gave colourless needles of the alcohol **2** (8 mg), m.p. 132-33°; $[\alpha]_D$ +64.4° (*c* 0.86 in $CHCl_3$). Anal. Calcd for $C_{29}H_{48}O$: C, 84.46; H, 11.65. Found: C, 84.36; H, 11.6%; no UV absorption above 210 nm; IR (KBr): 3510, 2950, 1600, 1470, 1390 and 1030 cm^{-1}; ^1H NMR (90 MHz): 0.72, 0.84, 0.91, 1.00, 1.20 (all methyls), 2.30 (1H, m, 25-H), 4.20 (1H, br m, $W_{1/2}$ = 20 Hz, 3a-H), 5.10 (1H, m, 23-H), 5.30 (1H, d, J = 6 Hz, 4-H).

Compounds from benzene extract. The light brown benzene extract (2 L) on concentration left a residue (2 g) which was chromatographed over a column of Si gel. The hexane eluate gave an oil (100 mg). The hexane-benzene (4:1) eluate gave a gummy substance (100 mg). The hexane-benzene (1:1) fractions gave a solid (150 mg), m.p. 135-36°;$[\alpha]_D$ −35°, which gavė +ve LB test (violet →blue→green) for steroids and was identified as β-sitosterol. The hexane-benzene (4:6) eluate gave a solid (30 mg), m.p. 80-81°, which was found identical in every respect with with those of ergosta-4,6,8(14),22-tetraen-3-one obtained from the hexane extract.

Compounds from methanol extract. The reddish-brown methanolic extract (2 L) was concentrated to a small volume (200 mL) and treated with basic lead acetate to remove tannins and other phenolic substances. The tannin free and deleaded extract on concentration left a solid (1.5 g) which on chromatography over a column of Si gel and eluting with chloroform-methanol (95:5) left a solid which on crystallisation from chloroform-methanol gave colourless shining plates (150 mg), m.p. 282-84°; $[\alpha]_D$ −40° (*c* 0.6 in pyridine). The solid gave +ve LB test for steroids and positive Molish test for glycoside. It was found identical in every respect with β-sitosterol-3β(+)-D-glucoside. No more useful compound was obtained from the column.

Acknowledsgement

One of us (S S R) is grateful to the UGC, New Delhi for the award of a fellowship under Faculty Improvement Programme.

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Indian Journal of Chemistry
Vol. 37B, April 1998, pp.387 - 390

Note

Benzopyrans: Part 39†—2-Amino-3-iminomethyl-1-benzopyran-4-ones do not function as heterodienes

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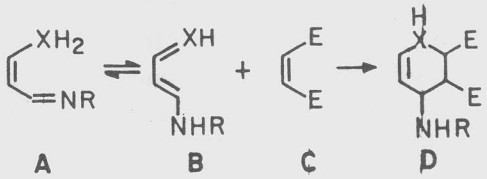
Received 10 October 1997; accepted 26 December 1997

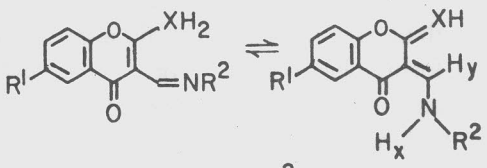
The hydrazone **3** (R^1=H and Me) in refluxing chloroform is converted by dimethyl acetylenedicarboxylate (DMAD) to the aminonitrile **12** whereas **3** and **4** when refluxed in DMF with either DMAD or *N*-phenylmaleimide undergo self-condensation to the diazocine **12** and pyranopyrimidine **14**, respectively instead of giving any [4+2]-cycloadduct.

β-Methyl-α,β-unsaturated imine (**A**, X=CH) undergoes through its enamine tautomer **B**, [4+2]cyclo-addition with a dienophile like **C** (**E**=electron withdrawing group) to give the cycloadduct **D** (Scheme I)[1]. 2-Methyl-3-iminomethyl-1-benzo-pyran-4-ones **1** and **2** incorporating the diene system **A** indeed give through their enamine tautomers **5** and **6** with *N*-phenylmaleimide (NPMI) the all carbon Diels-Alder adducts which have been converted to the xanthone system[2]. The title 1-benzopyran-4-ones (chromones) like **3** and **4** containing the azadiene system **A** (X=N) are nitrogen analogues of **1** and **2**, respectively. So these are likely to behave as 1-azadienes **7** and **8**, akin to *o*-quinone methide imines, in unergoing hetero Diels-Alder cycloaddition[3] with NPMI and dimethyl acetylenedicarboxylate (DMAD) to give respectively the adducts **9** and **10** which may be converted into 4-azaxanthone system[4-6]. That this contention was belied and the substrates **3** and **4** gave other products is reported in this note.

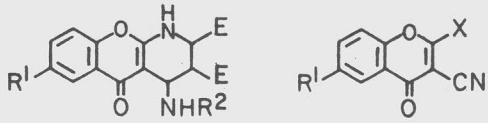
Unlike the initial 1,2-addition of phenyl-



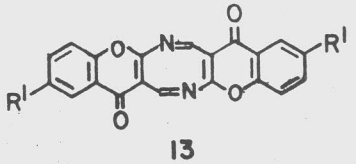
Scheme I



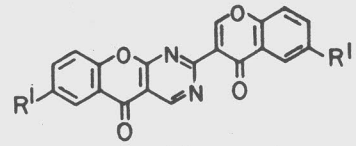
	X	R^2	
1 :	CH	NMe_2	: 5
2 :	CH	C_6H_4Me-*p*	: 6
3 :	N	NMe_2	: 7
4 :	N	C_6H_4Me-*p*	: 8



9 : EE = CONPhCO 11 : X = H
10 : E = CO_2Me 12 : X = NH_2



13



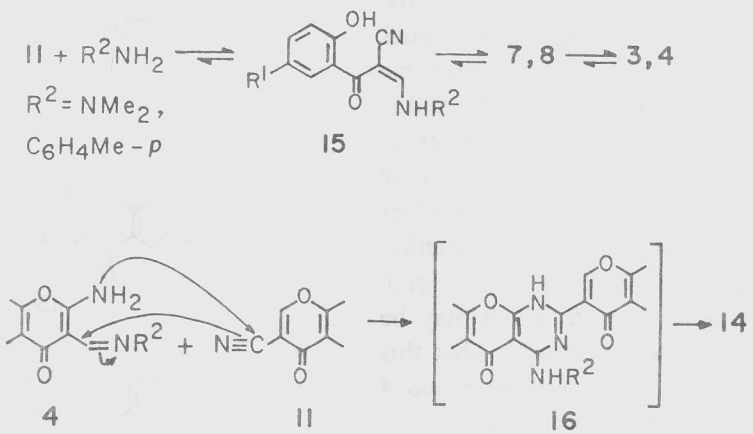
14

For
1 – 14 : a, R^1 = H ; b, R^1 = Me

†Part 38: Ghosh C K, Bhattacharyya S, Ghoshal N & Achari B, *J Chem Res*, submitted.

hydrazine to the nitrile function of chromone-3-nitrile **11** in ethanolic medium[6], refluxing benzene induces its initial 1,4-addition producing ultimately the phenylhydrazone derivative of 2-amino- 4 -oxo- 4*H*-1-benzopyran-3-carboxaldehyde[7]. Cognate preparation of the 2-aminochromone derivatives **7** and **8** by reacting the nitrile **11** with 1,1-dimethylhydrazine and *p*-toluidine respectively in boiling benzene was feasible. Here the nucleophile R^2NH_2 undergoes 1,4-addition to **11** with concomitant opening of the pyran ring to afford the acrylonitrile derivative **12**; cyclisation of the latter to **13** followed by a hydrogen shift forming **3** and **4** (Scheme II). Refluxing an ethanolic solution of 1,1-dimethylhydrazine with an equivalent amount of either the appropriate 2-amino-3-formylchromone[4] or 4-oxo-4*H*-1-benzopyran-3-aldoxime[4] also resulted in hydrazone **3**. IR stretching frequencies at *ca* 3200 and 3060 cm^{-1} indicate the presence of amino group in **3** but the amino protons are not detected in its ^1H NMR spectrum. Mass spectrum, however, was compatible with the assigned structure **3** (*vide* Experimental). The IR spectrum of **4** in KBr pellet shows an intense peak at 2210 cm^{-1} attributable to the presence of a cyano group, and ^1H NMR spectra indicates the compound to exist solely in its enamine tautomeric form **8** in its chloroform solution, the spectral pattern for its =CHNHAr grouping resembling that exhibited by the anilinomethylene grouping in the 1:2-adduct of 3-formylchromone and aniline[8]. So it is evident that compound **4**, depending on the conditions, can also exist solely or partially in its two other tautomeric forms **8** and **12**.

As the aminochromone **4** remains exclusively in the cisoid azabutadiene form **8** in chloroform solution, this substrate as well as its analogue **3** was first treated with DMAD and NPMI separately in refluxing chloroform. Under this reaction condition DMAD brought about elimination of dimethylamine[9] from the hydrazone **3** to afford the aminonitrile **12** whereas both the substrates **3** and **4** remained unreactive towards NPMI. When the above substrates were heated with either of the aforesaid dienophiles under reflux in dimethylformamide (DMF), **3** underwent self-condensation to the diazocine **13**[10] and **4** to the pyranopyrimidine **14**[11], the formation of any of the cycloadducts **9** and **10** being completely excluded. In refluxing DMF even the dedimethylamination of **3** by DMAD was predominated over by self-condensation of the former. Formation of **13** involves nucleophilic 1,2-addition of NH_2 group of one molecule of **3** to the $CH{=}NNMe_2$ group of its second molecule and subsequent elimination of two molecules of dimethylhydrazine. When heated under reflux in DMF, the Schiff base **4** reverses back through the tautomeric forms **8** and **15** ($R^2{=}C_6H_4Me$-*p*) to its precursor nitrile **11** (Scheme



Scheme II

II); the aminochromone **4**, then condenses with the nitrile function of **11** and the resultant intermediate **16** by elimination of *p*-toluidine affords the pyrimidine **14** (Scheme II).

Experimental Section

The reported melting points are uncorrected. All the new compounds gave satisfactory C, H, N analyses. Light petroleum refers to the fraction, b.p. 40-60°.

2-Amino-3-(*N, N*-dimethylhydrazonomethyl)-chromone 3: Method A. A mixture of the nitrile **11** (10 mmoles) and 1,1-dimethylhydrazine (1.1 g, ~0.75 mL, 10 mmoles) dissolved in dry benzene (50 mL) was heated under reflux for 4 hr. The reaction mixture was cooled, the deposited solid filtered off and crystallised from chloroform-light petroleum to afford the chromone **3**.

3a: Yield 62%, m.p. 216°; IR (KBr): 3200, 3062, (NH_2), 1662 (CO), 1607 (C=N) cm^{-1}; ^1H NMR: δ 8.24 (1H, dd, *J*=8, 2Hz, H-5), 8.08 (1H, s, CH=N), 7.64 -7.24 (3H, m, H-6, 7, 8) and 2.86 (6H, s, NMe_2); MS: m/z 231 (M^+, 18%), 187 (M-NMe_2, 100), 161 (187-CN, 11), 121 (HOC_6H_4CO, 44).

3b: Yield 69%, m.p. 212°; IR (KBr): 3222, 3050 (NH_2), 1638 (CO); ^1H NMR: δ 8.16 (1H, s, CH=N), 8.06 (1H, d, *J*~2Hz, H-5), 7.36 (1H, dd, *J*=8, 2Hz, H-7), 7.14 (1H, d, *J*=8 Hz, H-8), 2.86 (6H, s, NMe_2) and 2.42 (3H, s, Me-6); MS: m/z 245 (M^+, 40%), 201 (M-NMe_2, 100), 135 [$Me(OH)C_6H_3CO$, 30].

Method B. The appropriate 2-amino-3-formylchromone or 4-oxo-4*H*-1-benzopyran-3-aldoxime (10 mmoles) together with 1,1-dimethylhydrazine (10 mmoles) was refluxed in ethanol (100 mL) for 6 hr. The brownish yellow product deposited after concentration of the reaction mixture and subsequent cooling was collected by filtration and crystallised from chloroform-light petroleum to afford the hydrazone **3** identical with that obtained by method A. The yield of **3** by this method was around 50% from 2-amino-3-formylchromone and 60% from the aforementioned aldoxime.

2-Amino-3-(*p*-tolyliminomethyl)chromone 4. An equivalent mixture of **11** and *p*-toluidine was heated under reflux in benzene for 4 hr and then cooled when the title chromone **4** precipitated out. The compound **4a** (70%) melted at 211° and **4b** (74%) had m.p. 210°; IR (KBr): 3220 (NH), 3175 (OH), 2210 (CN), 1660 (CO) [for the tautomeric form **15** (R^2=C_6H_4Me-*p*)]; ^1H NMR ($CDCl_3$, 100 MHz): δ 12.44 (1H, d, *J*=13 Hz, H_X), 11.24 (1H, brs, =NH), 8.20 (1H, d, *J*=2Hz, H-5), 8.02 (1H, d, *J*=13 Hz, H_Y), 7.52-6.88 (6H, m, other ArH), 2.24 (3H, s, Me) and 2.20 (3H, s, Me) [compatible with the tautomeric form **8b**].

Treatment of the hydrazone 3 and Schiff base 4 with DMAD and NPMI: Method A. A mixture of **3** (1 mmole) and DMAD (0.1 mL) was heated under reflux in chloroform (50 mL) for 4-6 hr. The deposited solid was filtered and washed with chloroform to yield 2-amino-4-oxo-4*H*-1-benzopyran-3-carbonitrile **12** in 76-80% yield. The nitrile **12a** (76%) had m.p. 308° (decomp) (lit.[4], m.p. 310-11° decomp) and **12b** had m.p. >280°; IR (KBr): 3310, 3130 (NH_2), 2220 (CN), 1670 (CO), 1620 (C=C) cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 8.81 (2H, brs, exchangeable, NH_2), 7.67 (1H, ill split d, H-5), 7.46 (1H, ill split dd, *J*=8.2 Hz, H-7), 7.26 (1H, d, *J*=8.2 Hz, H-8) and 2.33 (3H, s, Me).

After treatment of **3** with NPMI and that of **4** with DMAD as well as with NPMI under similar conditions, the substrates **3** and **4** were recovered (~80%) unchanged.

Method B. An equivalent (10 mmoles) mixture of **3** and either DMAD or NPMI on being refluxed in DMF (25 mL) for 8-10 hr followed by usual work-up of the reaction mixture afforded 8,16-dioxo-8*H*,16*H*-diazocino[2,3-*b*:6,7-*b'*]bis[1]benzopyran **13** in 40-50% yield. The diazocine **13a**, m.p. 220-223° (lit.[10], m.p. 210°) and **13b**, m.p. 230° (decomp) (lit.[10], m.p. 215°) were identical (IR, ^1H NMR and mass spectra) with the respective authentic samples[10]. The Schiff base **4** on similar treatment with either NPMI or DMAD afforded 5-oxo-5*H*-2-(4-oxo-4*H*-1- benzopyraan-3-yl)[1]benzopyrano[2,3-*d*]pyrimidine **14** in 70-75% yield. The product **14a**, m.p. 245° (lit.[11], m.p. 240°) and **14b**, m.p. 290° (lit.[11], m.p. 297°) were identical (IR, ^1H NMR and mass spectra) with the respective authentic samples[11].

Acknowledgement

Financial support for this work from CSIR, New Delhi is gratefully acknowledged.

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Indian Journal of Chemistry
Vol. 37B, April 1998, pp.391 - 393

Note

Synthesis of isoetoposide and isoteniposide†

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Received 28 April 1997; accepted 6 October 1997

Condensation of glucosides of podophyllotoxin **4** and 4′-demethylpodophyllotoxin **5** with diethyl acetal in dry nitromethane and thiophene-2-carboxaldehyde yield the corresponding acetals namely, isoetoposide methyl ether **6** , isoetoposide **7** and isoteniposide **8**.

The aryl tetralinlignans of Podophyllum species have acquired considerable importance because of their cytotoxic and antitumor activity[1]. Podophyllotoxin **1** and some other structurally closely related lignans and lignan glucosides[2] isolated from the roots/rhizomes of *Podophyllum emodi* or *P. hexandrum* exert a powerful and specific inhibition of mitosis by stopping cell division in early metaphase[3]. Evaluated with systemic application as tumor damaging agents these natural products failed to act satisfactorily in clinical trials due to non specific toxic side effects[4].

It was observed that among all the glucoside derivatives prepared those belonging to 4′-demethyl series proved to be clinically most effective. The unique configuration of 4′-demethyl series at C-1, C-2, C-3 and C-4 with its highly strained transfused (γ-lactone system gives them the antimitotic and tumor damaging activity. Thus all these clinically effective compounds belong to epi series. We report herein the synthesis of isoetoposide and isoteniposide: cyclic acetals of normal series.

Glucosides of podophyllotoxin **4** and 4′-demethylpodophyllotoxin **5** series were isolated from American and Indian species by Stoll *et al.*[7] Keeping in view that the acetals of glucoside of 4′-demethylepipodophyllotoxin such as etoposide and teniposide (cyclic derivatives of epi series) show strong anticancer activity it was thought worthwhile to synthesise the corresponding acetals of the naturally occurring glucosides of podophyllotoxin and 4′-demethylpodophyllotoxin to yield isoetoposide **7** and isoteniposide **8** (Scheme I)..

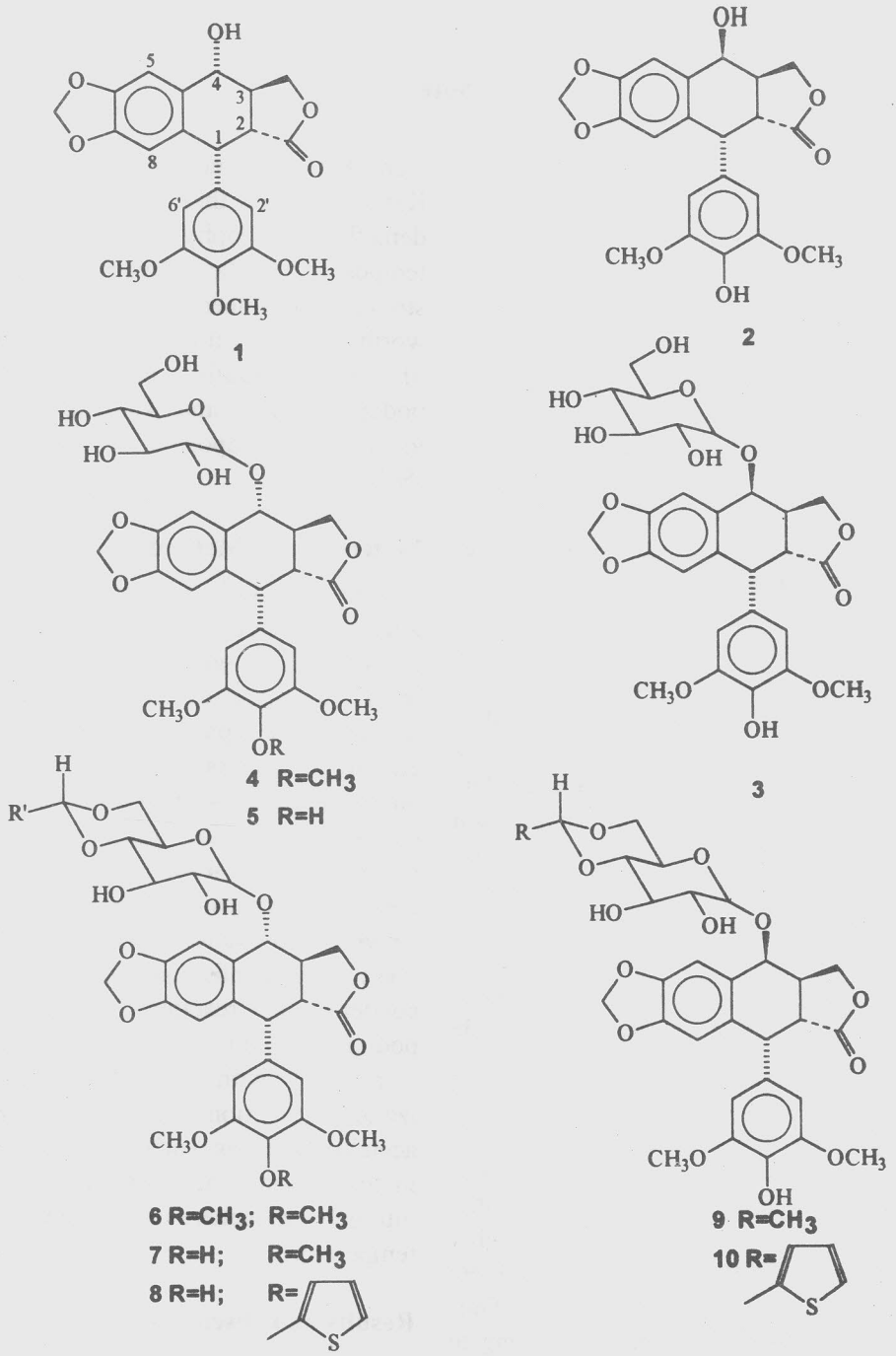
Materials and Methods

The mixture[9] of podophyllotoxin-4-O-β-D-glucopyranoside **4** and 4′-demethylpodophyllotoxin-4-O-β-D-glucopyranoside **5** was obtained from the marc of podophyllotoxin resin. The mixture was separated on silica gel column by elution with chloroform-methanol (95:5) to give podophyllotoxin-4-O-β-D-glucopyranoside followed by elution with chloroform-methanol (85:15) mixture to yield 4′-demethylpodophyllotoxin-4-O-β-D-glucopyranoside. These glucosides have been converted to cyclic derivatives[10]. The synthesis of these compounds has been achieved by the condensation reaction of the glucosides of podophyllotoxin and 4′-demethylpodophyllotoxin respectively with liquid aldehyde. For transacetalization reaction the dimethyl acetal of acetaldehyde was reacted with respective glucoside in the presence of *p*-toluenesulphonic acid under nitrogen atmosphere using suitable solvent at room temperature.

Results and Discussion

This work has provided a method for the synthesis of cyclic acetals namely isoetoposide methyl ether **6**, isoetoposide **7** and isoteniposide **8**. The condensation reaction of glucosides usually took place on the C-4 and C-6 hydroxyls of the hexapyranose moiety to yield the acetals where the equatorial substituent predominates almost exclusively (the isomer with an axial substituent is produced only in minimal amounts and is lost

†The compounds in the paper are covered under Indian Patent No. 1620/DEL/94 dated 14/12/94. Regional Research Laboratory contribution no. 2245.



Scheme I

during purification of the main product). A characteristic chemical shift[8] of the axial proton in the ^{1}H NMR spectrum of sugar acetals could be recognized especially with acetals of aromatic aldehydes.

Experimental Section

The marc comprising mainly of podophyllotoxin-β-D-glucopyranoside and 4′-demethylpodophyllotoxin-β-D-glucopyranoside, kaempferol, quercetin and other polyphenolics was subjected to separation by known methods[7,9]. ^{1}H NMR spectra were recorded on Varian T60 NMR spectrometer, mass spectra on Jeol DMS 300 spectrometer and IR spectra on a Shimadzu 435 IR spectrophotometer.

Podophyllotoxin-4 (4″, 6″-O-ethylidene-β-D-glucopyranoside); isoetoposide methyl ether 6. Powdered podophyllotoxin-4-O-β-D-glucopyranoside (1.2g, 0.002 mole) was suspended in 50 ml of dry nitromethane. Acetaldehyde diethylacetal (4 ml, 0.03mole) and *p*-toluenesulphonic acid (50 mg, 0.0003 mole) were added to it. The mixture was stirred under N_2, for 6 hr. The solution was diluted with chloroform (100 ml) and washed with water (3×25 mL). The organic phase was dried over sodium sulphate and solvent removed under vacuum yielding crude product (1.2 g). It was purified by column chromatography on silica gel (100 g) using chloroform-methanol (98:2) to give 1.1g of the pure material., m.p. 182-83°C (lit.[3] m.p. 160-64°C); $[\alpha]_D$ = –48° (c, 0.546, $CHC1_3$); –55.60° (c, 0.5, MeOH); IR (KBr):3482(OH), 1776 (γ-lactone), 1590, 1506, 1484 cm^{-1} (arom.C=C); ^{1}HNMR ($CDC1_3$): 7.20 (s, 1H, C_5-H), 6.45 (s, 1H, C_8-H), 6.30 (s, 2H C'_2 and C'_6-H), 5.95 (s, 2H, -O-CH_2-O), 4.8-3.8(2m,12H, H-4, H-1 & H-3aα, 3aβ embedded in 7 glucosidic protons and one acetal proton), 3.75 & 3.7 (2s, 9H, 3-OCH_3) 2.8 (m, 2H C_2 & C_3-H), 1.30 (d, *J*=5-Hz 3H, CH_3-CH<); Mass: M^+ 602.

4′-Demethylpodophyllotoxin-4-(4″, 6″-O-ethylidene-β-D-glucopyranoside); isoetoposide 7. Dried 4′-demethylpodophyllotoxin-4-O-β-D-glucopyranoside (1.2 g, 0.002 mole) was converted to its acetal which was purified by column chromatography over silica gel (90 g) using chloroforn-methanol (95:5) as eluant and recrystallised from methanol to give pure product (0.82 g), m.p. 174-75°C $[\alpha]_D$ -60° (c, 0.59, $CHC1_3$); –60° (c, 0.47,MeOH); IR (KBr): 3448(OH); 1772 (γ-lactone); 1614, 1512, 1482 cm^{-1}(arom. C=C); ^{1}HNMR ($CDC1_3$): 7.18(s, 1H C_5-H), 6.42(s, 1H C_8-H), 6.30 (s, 2H C_2'- & C_{61}-H), 5.95 (s, 2H, –O–CH_2–O–) 4.75-3.8 (2m, 12H, H-4, H-1 & H-3aα, 3aβ embedded in 7 glucosidic protons and one acetal proton), 3.68 (s, 6H, 2-OCH_3), 1.28 (d, *J*=4.5Hz, 3H CH_3–CH<O; Mass:M^+ 588.

4′-Demethylpodophyllotoxin 4-(4″, 6″-O-thenylidene-β-D-glucopyranoside); isoetoposide 8. Dried 4′-demethylpodophyllotoxin-4-O-β-D-glucopyranoside (0.825 g, 0.0015 mole) was suspended in thiophene carboxaldehyde (8mL) and anhydrous zinc chloride (400 mg). The product after usual work up was purified by column chromatography over silica gel (90 g) using chloroform-methanol (95:5) as eluant and recrystallised from methanol to give pure product (700mg), m.p. 315-20°C (lit.[3] m.p. 274-77°C); $[\alpha]_D$= –55.4° [c, 0.54, MeOH], IR(KBr): 3440(OH); 1770 (γ-lactone); 1610, 1510 and 1470 cm^{-1} (arom. C=C); ^{1}HNMR (DMSO-d_6): 7.7-6.8(m, 4H, H of thiophene ring and H at C_5-H), 6.56 (s, 1H, C_8-H), 6.22 (s, 2H C'_2-H & C'_6-H), 6.02(s, 2H. –O–CH_2–O–), 5.88 (s, 1H acetal H of thenylidene group), 4.85-3.65 (2m, 11H, H-4, H-1 & H-3aα, 3aβ embeded in seven glucosydic protons), 3.63 (s, 6H, 2OCH_3).

Biological activity

The anticancer screening of isoetoposide was performed at National Institute of Health, Maryland, USA. The compound did not show significant activity.

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Indian Journal of Chemistry
Vol. 37B, April 1998, pp.394 - 396

Note

Synthesis of peptides mediated by potassium salt of 1-hydroxy-7-azabenzotriazole

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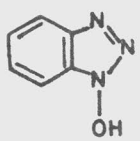
Received 24 February 1997; revised and accepted 1 December 1997

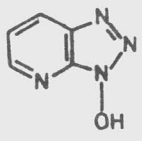
The potassium salt of 1-hydroxy-7-azabenzotriazole can be used along with acid chlorides of 9-fluorenylmethyloxycarbonylamino acids as coupling agents in peptide bond formation. The acylation reactions are rapid and efficient and can be carried out in an organic medium. The yields as well as purity of the peptides are satisfactory.

Ever since 1-hydroxybenzotriazole (HOBt) was suggested as a peptide coupling additive by Konig *et al.*[1], most common methods for peptide bond formation are carried out in its presence. It is used either in combination with a carbodiimide [dicyclohexylcarbodiimide (DCC) or *N,N*-diisopropylcarbodiimide (DIPCDI) or 1-ethyl-3-(3′-(dimethylamino)propyl)-carbodiimide (EDC)] or another coupling agent (active esters such as 2,4,5-trichlorophenyl or pentachlorophenyl or pentafluorophenyl or *O*-nitrophenyl ester) or built into a stand alone reagent [such as 1-benzotriazolyloxytris(dimethylamino) phosphonium- hexafluorophosphate (BOP), or benzotriazole-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate] or as an analogous uronium salt [*O*-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluoro phosphate, HBTU]. Such additives are known to inhibit several side reactions and reduce racemization.

Recently, Carpino[2] suggested the use of 1-hydroxy-7-azabenzotriazole (HOAt) in place of the auxiliary nucleophile HOBt. It is described as a more efficient additive which speeds up coupling processes and reduces the loss of chiral integrity. It incorporates within a single molecule, both key elements of the 1:1 mixture of HOBt and a tertiary amine which is of greater catalytic effect than HOBt itself in couplings involving active ester[3]. Similar to HOBt, the use of HOAt could be governed by the formation or reactivity of an active ester intermediate. It was used along with EDC during coupling of Z- or Bzl-Phg/D-Phg/Phe-Val/Val with Val/Ala-OMe.HCl or Val/Ala-OMe in dimethylformamide[2]. It enhanced coupling yields in solution by about 6-32 times. Similar enhanced reactivity was shown by uronium salt [HATU, *O*-(7-azabenzotriazol-1-yl)-1,1,1,3-tetramethyluronium hexafluorophosphate] compared to its HOBt analogue HBTU[4].



HOBt



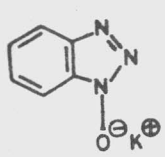
HOAt

Later, Carpino and co-workers[5] demonstrated the superiority of HOAt and its corresponding uronium {HATU, HAPyU [*O*-(7-azabenzotriazol-1-yl)-1,1,3,3-bis(tetramethylene)uronium hexafluorophosphate], HAPipU [*O*-(7-azabenzotriazol-1-yl)-1,1,3,3- bis (pentamethylene) uronium hexafluorophosphate], HAMDU [*O*-(7-azabenzotriazol-1-yl) - 1,3-dimethyl-1,3-dimethyleneuronium hexafluorophosphate] or HAMTU [*O*- (7-azabenzotriazol-1-yl) -1,3-dimethyl-1,3-trimethyleneuronium hexa-fluorophosphate]} and phosphonium {AOP[7-azabenzotriazol-1-yloxytris-(dimethylamino)phosphonium hexafluorophosphate] or PyAOP [7-azabenzotriazol-1-yloxytris (pyrrolidino)-phosphonium hexafluorophosphate]} salts to their benzotriazole analogues {HBTU, PyBOP [benzotriazol-1-yloxytris (pyrrolidino) phosphonium hexafluorophosphate] or PyBrOP [bromoxtris (pyrrolidino) phosphonium hexafluorophosphate] in solid phase peptide synthesis, thereby making possible the automated synthesis of peptides containing hindered amino acids. Thus, several syntheses of fragment 65-74 (Val-

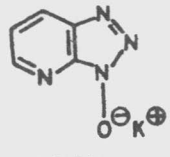
Gln-Ala-Ala-Ile-Asp-Tyr-Ile-Asn-Gly-NH_2) of the acyl carrier protein were made. Polyethylene glycol-polystyrene (PEG-PS)-resin containing a peptide amide linker derived from 5-[4-(Fmoc)-aminomethyl-3,5-dimethoxyphenoxy]valeric acid (PAL) was used as a solid support along with a continuous-flow Millipore 9050 plus synthesizer.

Based on the model studies involving the 2 hr couplings of Fmoc-Phe-Ser(OBu^t)-OH onto H-Pro-PAL-PEG-PS-resin, Carpino *et al.*[6], also demonstrated that HOAt, HATU, HAPyU, AOP are more effective in avoiding racemization in solid phase peptide segment coupling process than their benzotriazole analogues.

Acylation reactions using acid chlorides require a basic reaction environment. Either the conditions similar to Schotten-Baumann type reactions or nonaqueous media with an additional mole of an amino acid ester or an organic base has been employed[7]. Otherwise, the condensation reactions used to be incomplete. In this preliminary communication, the use of potassium salt of 1-hydroxy-7-azabenzotriazole (KOAt) instead of potassium salt of 1-hydroxybenzotriazole[8] (KOBt) is described. It is found that condensation of acid chlorides of 9-fluorenylmethyloxycarbonylamino acids[9,10] can be conveniently carried out in the presence of KOAt.



KOBt



KOAt

No additional base need be added. In addition, an organic solvent can be used as the solvent medium instead of biphasic system containing water. The physical data of the various dipeptide esters prepared by this procedure are given in Table I. A typical coupling is given in experimental procedure. The *C*-methylene doublets and the methyl ester singlets of ^{1}H NMR spectra[11] of the protected diastereomeric peptides, Fmoc-L-Phg-L-Phe-OMe [δ 3.17 (d, $CHCH_2C_6H_5$), 3.64 (s,OCH_3)] and Fmoc-D-Phg-L-Phe-OMe [δ 2.98 (d, $CHCH_2C_6H_5$), 3.73 (s, OCH_3)] prepared by this procedure clearly demonstrate that the coupling reactions mediated by KOAt are free from racemization. The application of this methodology has since been extended to the synthesis of β-casomorphin and these results will be published later.

Table I—Protected peptide esters

Sl No.	Name of the Peptide	Yield (%)	m.p. °C	R_f value R_fA^*	$R_fB^{\#\#}$	$[\alpha]_D^{25}$	Mol. formula	Found % (Calcd) C	H	N
1	Fmoc-Phe-Leu-OBzl	86	155-57	0.80	0.71	– 24.3° (c 1, CH_2Cl_2)	$C_{37}H_{38}N_2O_5$	75.12 (75.25	6.51 6.4	4.66 4.74)
2	Fmoc-Aib-Gly-OBzl	87	126-27	0.85	0.76	—	$C_{28}H_{28}N_2O_5$	72.00 (71.18	5.60 5.92	6.10 5.92)
3	Fmoc-Val-Gly-OBzl	88	184-86	0.80	0.68	+ 32.2° (c 1, CH_2Cl_2)	$C_{29}H_{30}N_2O_5$	72.10 (71.60	6.07 6.17	5.81 5.76)
4	Fmoc-Aib-Aib-OBzl	82	133-35	0.76	0.69	—	$C_{30}H_{32}N_2O_5$	72.12 (72.00	6.51 6.40	5.50 5.60)
5	Fmoc-Met-Val-OMe	71	90-92	0.94	0.83	– 12° (c 1, EtOH)	$C_{26}H_{32}N_2O_5$	64.43 (64.46	6.63 6.61	5.79 5.78)
6	Fmoc-Phg-Phe-OMe	82	193-95	0.79	0.73	+ 24° (c 1, CH_2Cl_2)	$C_{33}H_{30}N_2O_5$	74.13 (74.16	5.64 5.61	5.21 5.24)
7	Fmoc-D-Phg-Phe-OMe	81	192-94	0.80	0.76	+ 24° (c 1, CH_2Cl_2)	$C_{33}H_{30}N_2O_5$	74.19 (74.16	5.59 5.61	5.22 5.24)
8	Fmoc-Ile-Gly-OEt	79	111-13	0.93	0.61	– 32° (c 0.5, DMF)	$C_{25}H_{30}N_2O_5$	68.42 (68.49	6.88 6.84	6.41 6.39)
9	Fmoc-Phg-Gly-OEt	70	181-83	0.83	0.94	+ 56° (c 0.5, MeOH)	$C_{27}H_{26}N_2O_5$	70.71 (70.74	5.69 5.67	6.06 6.11)

*A = chloroform - methanol (9:1); ##B = chloroform - methanol - acetic acid (40:2:1); amino acid used, except Gly & Aib are of L-configuration, unless otherwise specified. Phg stands for phenylglycine

Conclusions

Racemization-free, rapid and efficient coupling reactions can be accomplished in a single organic phase using Fmoc-amino acid chloride/KOAt. The reaction is carried out in a single phase and therefore the scale-up as well as the working-up of the products are simple. The yield (70 to 88%) as well as purity of the final peptide are satisfactory.

The advantages of KOAt over KOBt for the synthesis of peptides enriched with hindered amino acids such as α-aminoisobutyric acid (Aib) and its use in solid phase peptide synthesis are being demonstrated. Further, the utility of Fmoc protected peptide acid chlorides in segment coupling is being explored.

Experimental Section

KOAt. HOAt (1.36 g, 10 mmoles) was added to an aqueous methanolic solution (6 mL) of potassium carbonate (0.7 g, 5 mmoles). After the evolution of CO_2 gas ceased, the solution was evaporated to dryness *in vacuo* to get crystalline solid. It was recrystallised from hot methanol-ether, yield 1.65 g (96%).

Fmoc-amino acid chlorides: General procedure. To the Fmoc-amino acid (1 mmole) suspended in 5 mL of CH_2Cl_2, 1 mL of $SOCl_2$ was added and the mixture stirred for about 24 hr at room temperature under dry conditions. Evaporation *in vacuo* followed by addition of CH_2Cl_2 and re-evaporation 2-3 times gave an oil or a solid free of excess $SOCl_2$. The residue was dissolved in little ether or CH_2Cl_2 and hexane was added. The resulting crystals were filtered (yield 85-90%) and dried.

Coupling of Fmoc-amino acid chloride with amino acid ester salt in the presence of KOAt : General Procedure. To a solution of an amino acid ester hydrochloride (1 mmole) and KOAt (1 mmole) in CH_2Cl_2 (3 mL) was added a solution of Fmoc-amino acid chloride (1 mmole) treated with KOAt (1 mmole) in CH_2Cl_2 (3 mL), and the reaction mixture stirred for 2-5 min at room temperature. The completion of the reaction was monitored on TLC. The mixture was then washed thrice with 5 mL portions of 5% HCl and water, and then dried over anhydrous sodium sulphate. Evaporation of solvent *in vacuo*, and recrystallisation of residue from suitable solvent gave the product in good yield. The purity of the corresponding free peptide after the removal of Fmoc group by using 4-(aminomethyl)piperidine was checked by RP-HPLC [Delta pack C-18 column (3.9 mm × 300 mm), flow rate : 1 mL/min; UV monitoring at 254 nm; eluant : H_2O and acetonitrile (62%) containing 0.1% trifluoroacetic acid] and found to be >96% pure.

Acknowledgement

Authors are thankful to the CSIR and to the UGC-DRS, New Delhi for the support of this work and to Prof. Louis A Carpino of University of Massachusetts for providing a sample of HOAt. We thank Prof. K M Sivanandaiah of our department for useful discussions. One of the authors (HNG) thanks Bangalore University for the award of a fellowship.

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Indian Journal of Chemistry
Vol. 37B, April 1998, pp.397 - 398

Note

Large scale synthesis of dimethyl 1,3-acetonedicarboxylate

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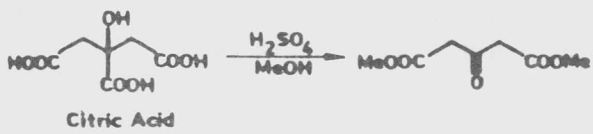
Received 26 March 1997; accepted 20 August 1997

Synthesis of dimethyl 1,3-acetonedicarboxylate from citric acid and sulfuric acid is described.

Dimethyl 1,3-acetonedicarboxylate 1 (DMAD) is a useful building block for the synthesis of various polycyclic and heterocyclic compounds For example, DMAD and diethyl 1,3- acetonedicarboxylate (DEAD) have been extensively used in the Weiss-Cook condensation reaction with various 1,2-dicarbonyl compounds, either at acidic or alkaline *p*H, to form *cis*-bicyclo[3.3.0]octane-3,7-dione derivatives in good yield[1]. DEAD undergoes self condensation in the presence of ethyl chloroacetate and magnesium (as catalyst) to furnish dihydroxy-homophthalate derivatives[2]. The diester **1** undergoes condensation reaction with salicylaldehyde derivatives in the presence of piperidine to form the biscoumarins[3].

In connection with our interest in polyquinane synthesis we needed large quantities of **1**. The price of this material[4] forced us to develop a simple and large scale preparative method. A perusal of literature revealed that acetonedicarboxylic acid is prepared from citric acid using fuming sulfuric acid[5]. Later on, the diacid was converted into the corresponding ethyl ester by treatment with absolute ethanol using sulfuric acid as catalyst[6]. Since this procedure requires large quantities of fuming sulfuric acid and isolation of the unstable diacid intermediate, it is desirable to have an alternate method. In this regard we are able to prepare **1** in 200 g quantities quite easily by decarboxylation of citric acid[7].

Reaction of citric acid with concentrated sulfuric acid at 30°C gave 1,3- acetonedicarboxylic acid which was esterified *in situ* using methanol as solvent. The operational simplicity of this procedure combined with cost of the starting material makes this method economically viable for large scale preparation of DMAD. We have repeated this reaction several times and found that **1** can be prepared routinely in 100-200 g quantities.



Citric Acid

Experimental Section

Procedure. In a 5 litre three necked round-bottomed flask equipped with mechanical stirrer and gas outlet was added 2,152 g (21.9 moles) of concentrated sulfuric acid. Thereafter, 420 g (2.18 moles) of citric acid monohydrate was added in small portions to avoid foaming. The reaction mixture was stirred for 3 hr maintaining the temperature at 20-25°C. Later on, the temperature was raised to 45°C using hot water-bath and the stirring continued for another 6 hr to complete decarbonylation[8]. After the reaction was over[9], the reaction mixture was cooled to 35-40°C and methanol (1,645 g, 51.4 moles) added to it slowly over a period of 2 hr and then stirring continued for another 1 hr. The reaction mixture was then divided into three equal portions and each portion was extracted with 1 L (500 mL × 2) of chloroform. Addition of ice cold water to the acid layer liberated 150-200 mL of chloroform. The combined organic layer was washed carefully with saturated bicarbonate solution, brine and dried ($MgSO_4$). Evaporation of the solvent under reduced pressure gave a light yellow oil which was further purified by vacuum distillation at 115-116°C/1 mm Hg to give 200 g (52%) of colourless liquid **1**[9]. The IR and ^1H NMR spectra of this material were identical to those of the commercial product. When this reaction was repeated with 1 mole of citric acid monohydrate, **1** was isolated in 70-75% yield[10].

Acknowledgement

Financial support from the Council of Scientific and Industrial Research, New Delhi is gratefully acknowledged. We thank RSIC, Mumbai for providing the spetral data. We also thank Professor K D Deodhar for useful discussions.

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Indian Journal of Chemistry
Vol. 37B, April 1998, pp.399 - 403

Note

Synthesis of functionaly substituted pyridine and thiophene derivatives

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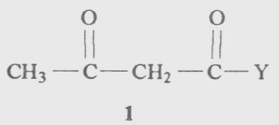
Received 18 April 1996; revised and accepted 2 December 1997

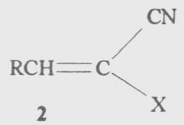
Acetoacetanilides **1** react with the ylidenenitriles **2** to give the substituted pyridines **4,5,10** and **11** respectively. Reaction of **1** with ethyl cyanoacetate and elemental sulphur in ethanolic-triethylamine yield 2-aminothiophenes **14**. Condensation of phenylhydrazone **15** with malononitrile affords pyridine **19**.

Diverse biological activities have been described for functionally substituted pyridines and fused pyridines. For example, pyridoxal phosphate plays an important role in metabolism as a coenzyme for a variety of biological transformations[1]. Nalidixic acid is bactericidal to most of common gram negative bacteria that cause urinary tract infection[2-4]. Moreover, several condensed pyridines have been used as antimalarials and antibactrials[5,6].

In the present note we report the new routes for the synthesis of substituted pyridines and thiophenes using β-ketoanilides **1** and the nitriles[7] **2** as starting components. Reaction of **1a,b** with arylidene-malononitriles **2a-c** in ethanolic-piperidine solution has been reported to give 3-acetylpyridones[8] **4**. Also **1** reacts with **2** in ethanolic-sodium ethoxide to give



1



2

1	Y
a	NHC_6H_5
b	$NHC_6H_4OCH_3$

2	R	X
a	C_6H_5	CN
b	$C_6H_4Cl(p)$	CN
c	$C_6H_4OCH_3(p)$	CN
d	C_6H_5	$CO_2C_2H_5$
e	$C_6H_4Cl(p)$	$CO_2C_2H_5$
f	$C_6H_4OCH_3(p)$	$CO_2C_2H_5$

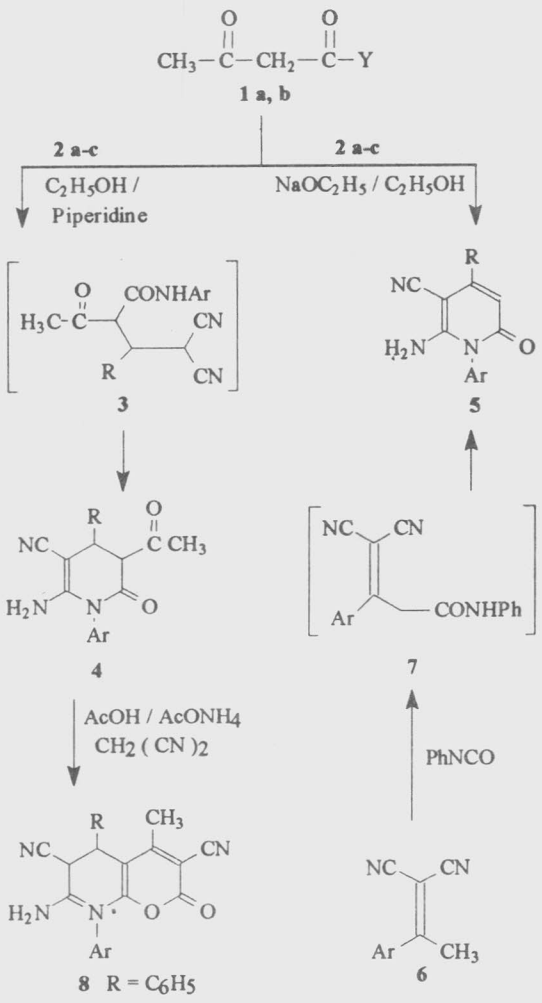
6-aminopyridones **5**. It is assumed to be formed by the addition of an active methylene in **1a,b** to the arylidenes **2a-c** to give the adduct **3**, which is cyclized to yield **4** (Scheme I). Compound **4** finally eliminates its acetyl group to form **5**. Elimination of acetyl group under similar conditions has already been reported[9].

Compounds **5a,b** were also obtained by reacting 1-arylethylidenemalononitriles **6** with phenyl isocyanate in dry dioxane containing catalytic amount of sodium metal[10]. The reactivity of acetyl group in **4** was studied. Thus, **4a** reacted with malononitrile in dry benzene containing catalytic amount of ammonium acetate and acetic acid to give **8** (cf. Scheme I), the structure of which was confirmed from its elemental analysis and IR spectra.

The behaviour of **1** towards ethyl arylidenecyanoacetates was investigated. Thus **1** reacted with **2d,e** to yield products, the type of which depends on the utilized reaction conditions. 3-Acetylpyridone **10** was obtained directly from the reaction of **1** with **2d,e** in ethanolic-piperidine (Scheme II).

On the other hand products with higher molecular weights were obtained from the same reactants on heating in ethanolic-sodium ethoxide. Structure **11** has been suggested for the reaction product, based on ^1H NMR spectra which clearly indicate the absence of acetyl function and the presence of olefinic protons in the product formed. Structure of **11** was also confirmed by its formation from the reaction of **10** with aromatic aldehydes in ethanolic-sodium ethoxide solution. One may assume a reaction pathway for the formation of **11** from **1** and **2d,e** that **1** reacts with **2** to yield a 1:1 adduct **9** followed by cyclization to **10**. Intermediate **10** then condenses with another molecule of aldehyde, which exists in equilibrium with **1** specially in aqueous basic medium[11] to give the chalcones **11** (Scheme II).

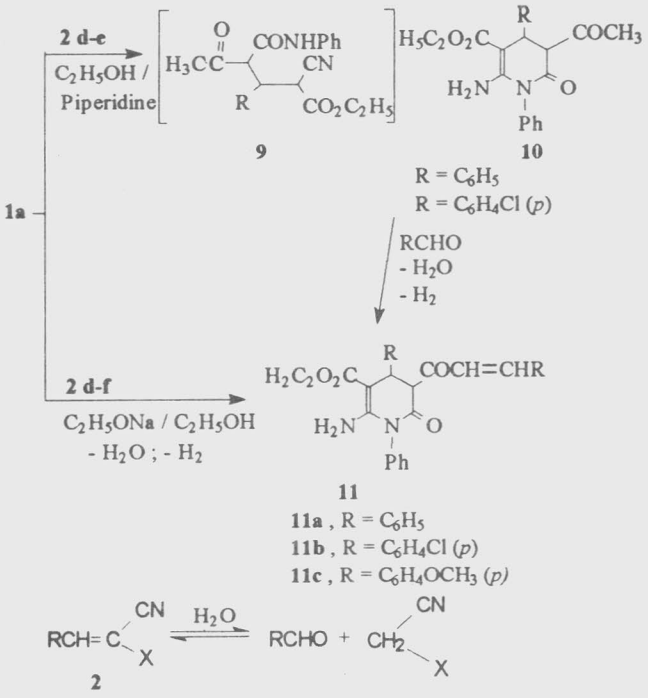
A mixture of **1a,b**, ethyl cyanoacetate and elemental sulphur when heated in ethanolic-triethylamine solution afforded 2-aminothiophenes **14a,b**. IR spectra of **14a,b** indicate clearly that the



4	R	Ar
a	C_6H_5	C_6H_5
b	$C_6H_4Cl(p)$	C_6H_5
c	$C_6H_4OCH_3(p)$	C_6H_5
d	$C_6H_4Cl(p)$	$C_6H_4OCH_3(p)$
e	$C_6H_4OCH_3(p)$	$C_6H_4OCH_3(p)$

5	R	Ar
a	C_6H_5	C_6H_5
b	$C_6H_4Cl(p)$	C_6H_5
c	$C_6H_4OCH_3(p)$	C_6H_5
d	C_6H_5	$C_6H_4OCH_3(p)$
e	$C_6H_4Cl(p)$	$C_6H_4OCH_3(p)$

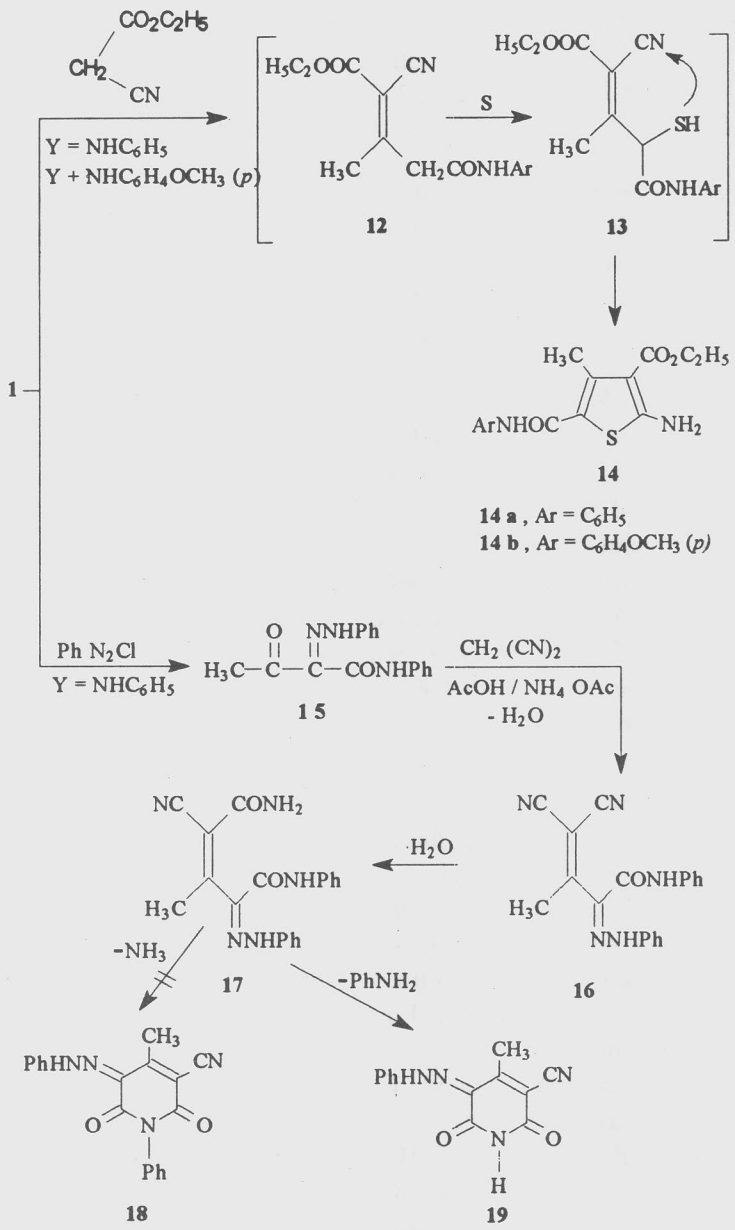
Scheme II



Scheme II

cyano functions are involved in the cyclization process and revealed the presence of amino groups. ^{1}H NMR spectrum of **14a** exhibits in addition to the aromatic protons, signals corresponding to NH, NH_2 and ester groups which is in good agreement with the proposed structures (cf. Scheme III). Formation of **14** is assumed to proceed via condensation of ethyl cyanoacetate with **1** to give an intermediate **12** which reacts with elemental sulphur to afford mercapto derivative **13**. Compound **13** then cyclizes to give product **14**.

Phenylhydrazone **15** when heated with malononitrile in dry benzene containing catalytic amount of ammonium acetate and acetic acid gave 3-cyano-2, 6-dioxo-4-methyl-1, 2, 5, 6-tetrahydropyridine-5-phenylhydrazone **19** and not the anticipated 3-cyano-2, 6-dioxo-4-methyl-1, 2, 5, 6-tetrahydro-1-phenylpyridine-5-phenylhydrazone **18**. The formation of **19** was established on the basis of its spectral (IR, ^{1}H NMR and mass) data. ^{1}H NMR spectrum of **19** exhibited a multiplet showing the presence of two NH groups. If this product was **18**[12] a singlet for NH would be expected. Further, the compound **19** was not identical with an authentic sample of **18** (m.p. and mixed m.p. and finger print region in the IR spectrum). A sequence of reaction of the formation of **19** is shown in Scheme III. Several



Scheme III

alkylheterocycles, such as 4-methylcoumarin[13], 4-methylpyridazines and 4-methyl-3-cyanopyridine-thione[14], reacted readily with cinnamonitriles **2** to yield fused heterocyclic systems, whereas the compound **19** failed to reach with the same reagents under a variety of drastic conditions.

Experimental Section

All melting points are uncorrected.IR spectra were recorded on a pye-unicam SP 1000 instrument; ^{1}H NMR spectra on EM 90-MHz spectrometer in DMSO-d_6 solution using TMS as internal standard (chemical shifts in δ, ppm), mass spectra on MS30 or MS9, (AEI) mass spectrometers operating at 70 eV. Microanalyses were carried out by the Microanalytical Data Unit at Cairo University.

3-Acetyl-6-amino-1, 4-diaryl-1, 2, 3, 4-tetrahydropyridine-5-carbonitriles (4a-e). A mixture of acetoacetanilides **1a** or **1b**-(0.01 mole) and **2a-c** in 50 mL of ethanol containing few drops of piperidine was refluxed for 5 hr. The mixture was then concentrated and cooled. The solid that separated was filtered off, dried and crystallized from ethanol to give **4a-e**. Compounds **4a-c** were previously obtained according to the literature procedure[8].

4b: Yield 2.2 g (60%), m.p. 195° (Found: C, 66.15; H, 3.53; N, 11.54. $C_{20}H_{13}ClN_3O_2$ requires C, 66.21; H, 3.61; N, 11.58%); IR: 3459, 3326, 3243 (NH_2)[15-18], 2184 (CN), 1715, 1708 (CO), 1645 (δNH_2).

4d: Yield 2.6 g (66%), m.p. 228° (Found: C, 63.81; H, 4.33; N, 10.36. $C_{21}H_{18}ClN_3O_3$ requires C, 63.72; H, 4.85; N, 10.62%); IR: 3450, 3320, 3310 (NH_2), 2195 (CN), 1725, 1700 (CO), 1665 (δNH_2).

4e: Yield 2.5 g (65%), m.p. 220° (Found: C, 67.62; H, 5.60; N, 10.53. $C_{22}H_{21}N_3O_4$ requires C, 67.51; H, 5.41; N, 10.74%); IR: 3450, 3350, 3255 (NH_2), 2198 (CN), 1718, 1700 (CO), 1638 (δNH_2).

6-Amino-1, 4-diaryl-2-oxo-1, 2-dihydropyridine 5-carbonitrile 5. Method A: A solution of **1a** or **1b** (0.01 mole) in abs. ethanol (50 mL) containing 0.23 g (0.01 mole) of finely divided sodium metal and 0.01 mole of the arylidenes **2a-c** was refluxed for 6 hr and then left to cool. The solution was then neutralized with dil. HCl. The products so formed were collected by filtration, crystallized from ethanol to give **5a-c**.

Method B: A mixture of **6a** or **6b** (0.01 mole), finely divided sodium metal (0.23 g, 0.01 mole), phenyl isocyanate (1 mL, 0.01 mole) in dry dioxane (40 mL) was refluxed for 4 hr. It was cooled and poured onto cold water and neutralized with dil. HCl. The precipitate was filtered off and crystallized from ethanol to give **5a-e** which were identified by comparison with authentic samples[10].

5c: Yield 1.9 g (60%), m.p. 205° (Found: C, 71.80; H, 4.82; N, 31.21. $C_{19}H_{15}N_3O_2$ requires C, 71.91; H, 4.76; N, 13.24%); IR: 3350, 3200 (NH_2), 2195 (CN), 1710 (CO), ^1H NMR: 3.65 (s, 3H, OCH_3), 6.75-7.80 (m, 9H, ArH and 1H, CH), 9.85 (s, 2H, NH_2)[10]; MS: m/z 317 (M^+).

5d: Yield 2.0 g (63%), m.p. 180° (Found: C, 72.11; H, 4.38; N, 13.51. $C_{19}H_{15}N_3O_2$ requires C, 71.91; H, 4.76; N, 13.24%); IR: 3350, 3250, 3200 (NH_2), 2210 (CN), 1710 (CO); ^1H NMR: 3.68 (s, 3H, OCH_3), 6.76-7.82 (m, 9H, ArH and 1H, CH), 10.2 (s, 2H, NH_2); MS: m/z 317 (M^+).

5e: Yield 2.2 g (62%), m.p. 175° (Found: C, 64.52; H, 4.33; N, 12.00. $C_{19}H_{14}ClN_3O_2$ requires C, 64.87; H, 4.01; N, 11.94%); IR: 3425-3230 (NH_2), 3195 (CN), 1695 (CO), 1640 (δNH_2).

7-Amino-4-methyl-2(*H*)-oxo-5, 8-diphenyl-5,6-dihydropyrano[2,3-*b*]pyridine-3, 6-dicarbonitrile 8. To a mixture of **4a** (0.01 mole), ammonium acetate (0.3 g) and acetic acid (0.5 mL) in dry benzene (50 mL), malononitrile (0.01 mole) was added. The reaction mixture was refluxed for 6 hr. The resulting solid product was filtered and crystallized from ethanol-DMF to give **8**, yield 2.3 g (60%), m.p. >300° (Found: C, 72.70; H, 4.43; N, 14.64. $C_{23}H_{16}N_4O_2$ requires C, 72.62; H, 4.24; N, 14.73%); IR: 3458, 3410, 3326 (NH_2), 2203 (CN), 1685 (CO), 1663 (δNH_2).

Ethyl 3-acetyl-6-amino-2-oxo-1-phenyl-1,2,3,4-tetrahydropyridine-5-carboxylates 10a,b. To a solution of **1a** (0.01 mole) in abs. ethanol (50 mL) containing 0.5 mL of piperidine **2d** or **2e** (0.01 mole) were added. The reaction mixture was refluxed for 1 hr, and cooled. The precipitate thus separated was filtered and crystallized from ethanol to give **10a,b**.

10a: Yield 2.5 (66%),m.p. 143° (Found: C, 69.65; H, 5.91; N, 7.35. $C_{22}H_{22}N_2O_4$ requires C, 69.83; H, 5.86; N, 7.40%); IR: 3450, 3330 (NH_2), 1730, 1720, 1650 (CO).

10b: Yield 3.3 g (80%), m.p. 130° (Found: C, 63.90; H, 5.01; N, 6.52. $C_{22}H_{21}ClN_2O_4$ requires C, 64.00; H, 5.13; N, 6.79%); IR: 3465, 3269 (NH_2), 1737, 1720, 1649 (CO); ^1H NMR: 1.2 (t, 3H, CH_3), 2.32 (s, 3H, CH_3), 4.2 (q, 2H, CH_2), 4.3 (s, 1H, pyridine H-4), 5.85 (s, 1H, pyridine H-3), 7.10-7.52 (m, 11H, 9H, ArH and 2H, NH_2).

3-Aryl-1-(6-amino-4-aryl-1, 2-dihydro-5-ethoxycarbonyl- 2 -oxo-1-phenylpyridine-3-yl)prop - 2-enone 11a-c. Method A: A suspension of **1a** (0.01 (mole) and **2d-f** (0.1 mole) in ethanol (50 mL) containing 0.01 mole of sodium metal were refluxed for 10 hr and then cooled. The reaction mixture was neutralized with HCl and the solid precipitated was filtered off, recrystallized from ethanol to give **11a-c.**

Method B: Compounds **11a,b** could also be prepared from **10a,b** and aromatic aldehydes and by usual work-up as reported in Method A.

11a: Yield 3.2 g (70%), m.p. 235° (Found: C, 74.66; H, 5.11; N, 6.34. $C_{29}H_{24}N_2O_4$ requires C, 74.98; H, 5.21; N, 6.03%); IR: 3400, 3350, 3200 (NH_2), 1725, 1685, 1675 (CO), 1665 (NH_2); MS: m/z 464 (M^+).

11b: Yield 3.2 g (60%), m.p. 252° (Found: C, 65.11; H, 4.45; N, 5.36. $C_{29}H_{22}Cl_2N_2O_4$ requires C, 65.30; H, 4.16: N, 5.25%); IR: 3350, 3200 (NH_2), 1680, 1670, 1665 (CO), 1655 (δNH_2); ^1H NMR:

1.13 (t, 3H, CH_3), 2.0 (s, 3H, CH_3), 4.16 (q, 2H, CH_2), 7.10 (d, *J*=9 Hz, 1H, CH), 7.40-7.76 (m, 13H, ArH), 8.20 (d, *J*=9 Hz, 1H, CH), 10.32 (s, 2H, NH_2).

11c: Yield 3.1 g (60%), m.p. 225° (Found: C, 71.23; H, 5.40; N, 5.12. $C_{31}H_{28}N_2O_6$ requires C, 70.98; H, 5.38; N, 5.34%); IR: 3360, 3330, 3250 (NH_2), 1700, 1685, 1675 (CO), 1660 (δNH_2), 1610 (C=C).

Ethyl 2-amino-5-arylcarboxyanilido-4-methyl-thiophene-3-carboxylates 14a-c. Equimolecular amounts of the anilide **1a** or **1b** (0.01 mole), ethyl cyanoacetate and elemental sulphur (0.01 mole) in ethanol (50 mL) were refluxed together with triethylamine (1 mL) for 1 hr. The solid obtained on cooling was filtered off and crystallized from ethanol to yield **14a,b**.

14a: Yield 2.1 g (70%) m.p. 172° (Found: C, 59.15; H, 5.47; N, 9.08. $C_{15}H_{16}N_2O_3S$ requires C, 59.19; H, 5.30; N, 9.20%); IR: 3480, 3350, 3275 (NH_2, NH), 1678 (CO), 1640 (δNH_2); ^1H NMR: 1.10-1.40 (t, 3H, CH_3), 2.48 (s, 3H, CH_3), 3.56-4.16 (q, 2H, CH_2), 6.8-7.04 (m, 7H, 5H, ArH and 2H, NH_2), 9.60 (s, 1H, NH); MS: m/z 305 (M^+).

14b:Yield 2.4 g (73%), m.p. 155° (Found: C, 57.21; H, 5.68; N, 8.54. $C_{16}H_{18}N_2O_4S$ requires C, 57.47; H, 5.43; N, 8.38%); IR: 3475, 3325 (NH_2, NH), 1675 (CO), 1660 (δNH_2); MS: m/z 334 (M^+).

3-Cyano-2, 6-dioxo-4-methyl-1, 2, 5, 6-tetrahydropyridine-5-phenylhydrazone 19. To a mixture of 2-acetyl-*N*-phenylethanamide-2-phenylhydrazone **15** (0.01 mole) in dry benzene (50 mL), 0.5 g of ammonium acetate and glacial acetic acid (8 mL) was added malononitrile (0.01 mole). The reaction mixture was refluxed for 6 hr. Evaporation of benzene left a solid product which was crystallized from DMF to give **19**, yield 1.6 g (63%), m.p. 265° (Found: C, 61.13; H, 4.24; N, 21.76. $C_{13}H_{10}N_4O_2$ requires C, 61.41; H, 3.96; N, 22.04%); IR: 3470, 3350 (NH), 2200 (CN), 1685, 1660 (CO), 1650 (C=N); ^1H NMR: 2.6 (s, 3H, CH_3), 7.36-7.52 (m, 5H, ArH), 7.6-7.8 (m, 2H, 2NH); MS: m/z 254 (M^+).

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Indian Journal of Chemistry
Vol. 37B, April 1998, pp.404 - 407

Note

Mercuration of bis[2-(*N*-benzylidene) phenyl]disulfides

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Received 14 July 1997; accepted 20 October 1997

The ligands **1** are obtained by condensing bis(2-aminophenyl)disulfide with *para*-substituted benzaldehydes. The mercuration of the ligands **1** to give the products **2** has been studied. The structural characterisation of the products **2** by IR and ^{1}H NMR spectra indicates that mercury is directed to the *para*-position of the *N*-phenyl ring.

The organometallic compounds of nontransition elements have received considerable attention in the last few years[1-8]. The organic chemistry of mercury and its biochemical and organometallic applications[3] have attracted special attention[2-8]. The use of organomercurials in the synthesis of other M-C σ-bonded organometallics have several advantages[6-8] over classical organolithium and Grignard reagents. The sites of mercuration of aromatics are electron population controlled and the stability of the products arise from the conformation of the substrate[4,7]. We have reported earlier the mercuration of a series of thioazomethines[7]. Herein we give an account on the regioselective mercuration of bis[2-(*N*-benzylidene)phenyl]disulfides.

Experimental Section

The solvents used in the reactions were of reagent grade and were dried by reported procedures[7]. $Hg(OAc)_2$ was purchased from Loba Chemie Indo Austranal Co., Bombay. All other chemicals were of reagent grade and were used as such. IR spectra (KBr) were recorded on a Perkin-Elmer 783 spectrophotometer; ^{1}H NMR spectra on a Varian XL 200 MHz FT NMR spectrometer in $CDCl_3$ for the ligands **1** and in DMSO-d_6 for mercurated products **2** using TMS as internal standard; and UV-Vis spectra on a Shimadzu UV-160A spectrophotometer. Elemental analyses were done by using a Perkin-Elmer 240C elemental analyser.

Bis[2-(*N*-benzylidene)phenyl]disulfide 1a. Benzaldehyde (1.13 g, 10.66 mmoles) was added to dry ethanolic solution (20 mL) of bis(2-aminophenyl)disulfide (1.20 g, 4.9 mmoles) and magnetically stirred for 1 hr. The orange yellow precipitate was filtered, washed with cold ethanol and dried over $CaCl_2$, yield 70%.

Compound **1b-e** were prepared similarly in 65-80% yields. The characterizations data of **1a-e** are given in Table I.

Bis[2-(*N*-benzylidene)(5-chloromercurio)phenyl]disulfide 2a. A solution of $Hg(OAc)_2$ (0.35 g, 1.1 mmoles) in dry MeOH (25 mL) was added dropwise to a solution of ligand **1a** (0.212 g, 0.5 mmole) in $CHCl_3$-MeOH (1:1 v/v, 20 mL). The mixture was stirred at controlled temperature (20-25^{0}C) for 40 hr. Very faint precipitate appeared and it was filtered off. LiCl (5 mmoles) was added to the filtrate, warmed for a few minutes and the resulting mixture was filtered, washed with ether and dried *in vacuo*. The product was recrystallised from DMSO-MeOH mixture to give **2a**, yield 55%.

Compound **2b-e** were prepared similarly in 50-70% yields. The characterization data of **2a-e** are given in Table I.

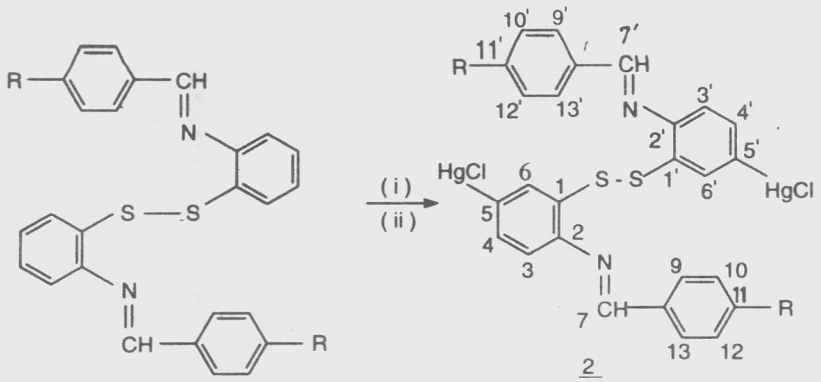
Results and Discussion

The Schiff bases and complexes described in this work are shown in Scheme I. The ligands **1** were synthesised in good yields by condensing *para*-substituted benzaldehydes with bis(2-aminophenyl)disulfide in alcohol. The chloromercuric derivatives **2** of the ligands **1** were obtained by the reaction $Hg(OAc)_2$ with the ligands **1** in 1:2 mole ratio followed by the addition of LiCl (cf. Table I).

IR spectra of **1** showed appearance of sharp strong band at 1600-1620 cm^{-1}, suggestive of νC=N mode[9]. In chloromercuric derivates **2** the position of νC=N in the IR spectra remains almost unshifted and is an indication of free azomethine group. The νC–S mode of the ligand[10] appears at 760-780 cm^{-1} which remains unshifted in mercurated products, and supports the presence of free S-centre. The

Table I—Characterization data of compounds **1a-e** and **2a-e**

Compd	Found (%) (Calcd)			m.p.	^{1}HNMR							
	C	H	N	^{0}C	3-(3'-)H (d)	4-(4'-)H	5-(5'-)H	6-(6'-)H	7-(7'-)H (s)	9,13-(9',13'-)H (d)	10,12-(10',12'-)H	R
1a	73.7	4.6	6.8	160	7.4	7.22 t	7.22 t	7.03d	8.43	7.92	7.67 t	---
	(73.6)	4.7	(6.6)									
1b	74.1	5.1	6.3	216	7.34	7.21 t	7.21 t	7.07 d	8.40	7.85	7.42 d	2.40 (CH_3)
	(74.3	5.3	6.20)									
1c	69.2	5.1	5.9	120	7.32	7.11 t	7.11 t	7.05 d	8.39	7.79	7.30 d	3.84 (OCH_3)
	(69.4	5.0	5.80)									
1d	63.4	3.5	5.8	170	7.48	7.24 t	7.24 t	7.10 d	8.45	8.05	7.71 d	---
	(63.3	3.7	5.7)									
1e	60.6	3.6	11.0	240	7.52	7.27 t	7.27 t	7.15 d	8.61	8.16	8.34 d	---
	(60.7	3.5	10.9)									
2a	35.0	2.1	3.0		7.49	7.55 d	-----	7.72 s	8.44	7.96	7.73 t	---
	(34.9	2.0	3.1)									
2b	36.6	2.0	3.1		7.45	7.51 d	-----	7.70 s	8.44	7.88	7.47 d	2.38 (CH_3)
	(36.4	2.2	3.0)									
2c	35.1	2.3	3.0		7.10	7.48 d	-----	7.68 s	8.42	7.83	7.38 d	3.85 (OCH_3)
	(35.2	2.3	2.9)									
2d	32.5	1.8	2.8		7.56	7.62 d	-----	7.78 s	8.49	8.09	7.83 d	---
	(32.4	1.7	2.9)									
2e	31.8	1.5	5.6		7.61	7.69 d	-----	7.84 s	8.68	8.22	8.44 d	



(i) Hg (OAc)2 / MeOH ; (ii) LiCl , H_2O
(a) R = H ; (b) R= Me ; (c) R = OMe ; (d) R = Cl ; (e) R = NO_2

Scheme I

νHg–Cl appears as a single sharp stretch[7] at 325-335 cm^{-1}.

The complexes are sparingly soluble in common organic solvents and the UV-VIS spectra are recorded in DMSO. The bands at 335-360 and 260-270 nm in UV spectra may be ascribed to $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions[11] in complexes and in ligand they are red shifted by 15-20 nm.

The site of mercuration is confirmed by ^{1}H NMR studies. All aromatic protons of the ligands **1** and chloromercuric derivatives **2** are unambiguously assigned on the basis of spin-spin structure and changes therein on substitution (Table I). The spectra of the ligands in $CDCl_3$ and the complexes in DMSO-d_6 are in complete agreement with the structures in which halves of the molecule are magnetically equivalent to each other (e.g., 6=6' etc.). The aromatic region of **1b-e** and **2b-e** displays symmetrical peaks characterising four spin AA' BB' system : 9-(9'-)H, 13-(13'-)H at 7.8-8.2 and 10-(10')H, 12-(12'-)H at 7.3-8.4 ppm. The signals due to 10-(10'-)H and 12-(12'-)H vary considerably with different substituents R in aromatic aldehyde. The signal movement is in accord with the inductive and electronic effects[7] of the group R. The -Me substitution shifts the signal upfield and the maximum shifting is observed in methoxy derivatives. The reverse effect is seen in nitro derivatives. The electron withdrawing character of $-NO_2$ shifts the signals considerably downfield, so these can only be assigned to the *C*-phenyl protons. The signals at higher field correspond to *N*-phenyl protons which is supported from the electron density calculation[12] and remain almost unperturbed on substitution in *C*-phenyl ring.

On mercuration *N*-phenyl protons are severely affected. The most significant feature is the loss of resonance due to 5-(5'-)H from the spectra and the appearance of new singlet due to 6-(6'-)H at very downfield position. The imine proton 7-(7'-)H expectedly appears at the most downfield position as a sharp singlet and moves slightly on either direction depending on the nature of substituents, R.

The regioselective mercuration is justified on the basis of electron density distribution and the stability of the product is conformation controlled[4,7]. The upfield position of the *N*-phenyl protons indicates the higher electron density of the *N*-phenyl as compared to the *C*-phenyl ring. This accounts the mercuration at *N*-phenyl ring and the conformational stability directs it to *para*-position of the *N*-phenyl ring.

Acknowledgement

Financial help from DST, New Delhi is gratefully acknowledged. We thank to Dr B B De, University of Nottingham, UK for ^{1}H NMR spectra.

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Indian Journal of Chemistry
Vol. 37B, April 1998, pp.408 - 410

Note

$MgI_2.Et_2O$/TMSCl induced catalytic isomerization of isospirostanes to furostenols†

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Received 15 May 1997; revised and accepted 27 November 1997

Magnesium iodide-ethereate and chlorotrimethyl-silane permit isomerization of isospirostanes to furostenols in acetic anhydride solution at boiling point in good yield.

For the convesion of spirostanic compound, diosgenin **1** (22-iso-5-spirostane-3β-ol) into 5,16-pregnadiene-3β-ol acetate 20-one **7**·known as 16-dehydropregnenolone acetate (16-DPA), a key intermediate for almost all the steroidal drugs including corticosteroids, sex hormones and oral contraceptives etc, it must be isomerized first to furostenol compound 5,20(22)-furostadiene-3β,26-diol diacetate **4** which on subsequent oxidation of the 20(22) double bond followed by hydrolysis and degradation furnishes 16-DPA[1,7,8]. Chemical transformations of spirostanic compounds also have importance towards the synthesis of plant growth-promoting substances including ecdysteroids and some furostenols having significant phytotoxicity[2]. The condition of the isomerization reaction reported by Marker *et al.*[3], involved the use of acetic anhydride at an elevated temperature and pressure, while the use of high boiling anhydrides as solvent led to lower yield of the product.

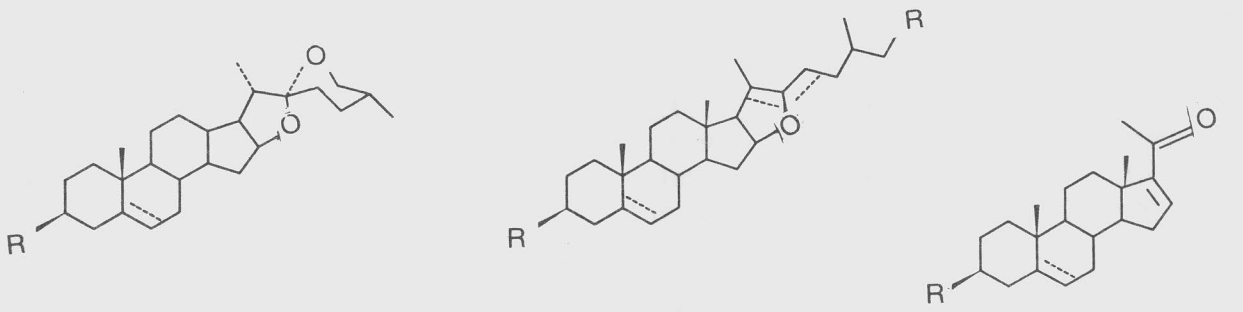
Recently, we have reported[1a] an improved process for the production of 16-DPA from diosgenin wherein the isomerization step has been achieved in a moderately boiling organic solvent using slight excess of the stoichiometric amount of acetic anhydride in a pressure reactor. The catalytic isomerization of isospirostane to furostenol was first studied by Gould *et al.*[4] and they observed that the conversion could be achieved at the boiling point of acetic anhydride in the presence of $AlCl_3$ or AcCl. Similar conditions using acetyl chloride also have been used for the transformation of $\Delta^{4,6}$-22-isospirostadiene-3-one to the corresponding 3-acetoxy-3,5,7-triene[5]. The poor yield of the desired isomerized product (33-40%) is the main disadvantage of all these methods. To improve the yield of the isomerised product, several other workers have reported[6-8] the isomerization of spirostanic compounds to furostenols in 70-84% yield by using various catalysts like pyridinium hydrochloride, pyridine and ammonium chloride or anhydrous titanium tetrachloride etc. However, to eliminate the use of toxic and costly catalyts like pyridine or pyridinium salts or titanium tetrachloride, the use of alternative non-toxic catalysts are desirable for this transformation. In the present method the non-toxicity and easy handling of the catalyts like magnesium iodide or chlorotrimethylsilane would make the method preferable to other catalystic methods. Indeed when the isospirostanes **1** and **2** were treated with either $MgI_2.Et_2O$-Ac_2O or TMSCl-Ac_2O system at the boiling point of acetic anhydride, isomerization to furostenols took place as desired. The favourable elimination of C-20 proton gave the stable furostenol, i.e. pseudodiosgenin diacetate **4** (67%) as the major product having C-20(22) double bond and the elimination of C-23 proton gave the other isomeric compound **5** (17%) having C-22(23) double bond as the minor one. Similarly, isospirostane **3** furnished furostenol **6** (58%) as the major isolable product for its eventual conversion to 16-DPA analogue **8**.

This isomerization of isospirostanes to furostenols is due to the possible attack by an acylonium ion[9-11] ($MeC^+=O$) facilitated by MgI_2 or TMSCl, on the nucleophillic pyran oxygen of the spirostanes **1-3** (Scheme I).

Experimental Section

Melting points were determined on a Mettler FP 62 instrument and are uncorrected. IR spectra were recorded on a Perkin-Elmer 237B spectrophotometer for solutions in chloroform; ^1H NMR spectra

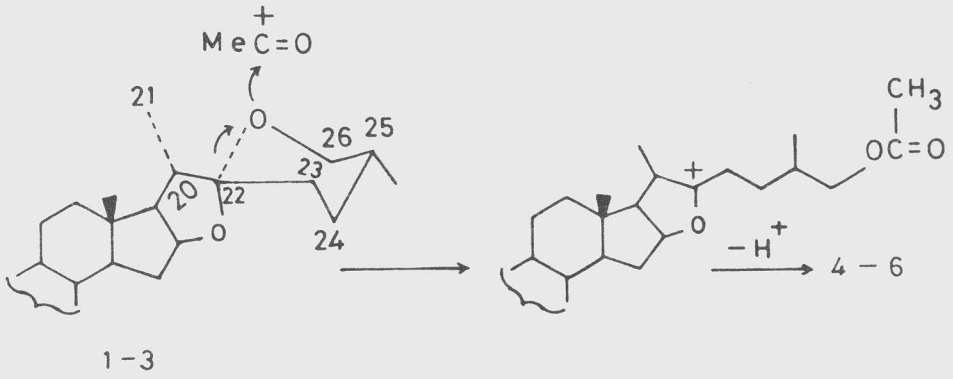
†A patent has been filed in India [ref 1(b)]



1 Δ^5, R = OH
2 Δ^5, R = OAc
3 R = OH

4 Δ^5, 20 (22), R = OAc
5 Δ^5, 22 (23), R = OAc
6 Δ^{20}, (22), R = OAc

7 Δ^5, R = OAc
8 R = OAc



Scheme I

in $CDCl_3$ on a Varian T-60 instrument and mass spectra on an INCOS 50 GC-MS instrument. TLC and preparative TLC were performed on silica gel (E Merck); plates were activated at 100°C for 1 hr.

Isomerization of isospirostanes to furostenols. General procedure. To a solution of the substrate **1-3** (0.5 mmole) in 4 mL of acetic anhydride was added freshly prepared colourless solution of magnesium iodide-ethereate[10] (10 mL) in dry diethyl ether or 5 mL of chlorotrimethylsilane. The reaction mixture was refluxed for a period of 4 hr. After completion of the reaction (TLC), the reaction mixture was quenched with cold water and extracted with hexane (3×150 mL). The organic extract was dried over anhydrous sodium sulphate and evaporated under reduced pressure to get a residue which was purified by chromatography to get the isomerized products **4-6**.

(a) **Isomerization of 1 or 2.** 1 g of Diosgenin **1** or its acetate **2** was isomerized as per general procedure using $MgI_2.Et_2O$ which after purification gave two products. The less polar compound **4** (800 mg) which is major was found to be identical with authentic furostenol pseudodiosgenin diacetate[1,7] in all respects [superimposable IR, ^1H NMR, mass spectral data and undepressed melting point (97-98°C)].

The more polar (minor) product was characterised as compound **5** (200 mg) which is a gum; Mass: m/z 498 (M^+), 438, 378, 362; IR: 1735 (two acetates), 1400, 1250 cm^{-1}; ^1H NMR: δ 1.1 (bs, 6H, methyl protons), 0.9 (bs, 6H, methyl protons), 2.1 (bs, 6H, acetate protons), 3.8 (d, 2H, *J*=7.5 Hz, 26-methylene protons), 5.3 (m, 2H, olefinic protons).

Similarly, compounds **1** and **2** (1 g) furnished the compound **4** (700 mg) and the compound **5** (150 mg) respectively when chlorotrimethylsilane (TMSCl) was used in place of magnesium iodide ethereate.

(b) **Isomerization of sarsasapogenin 3**. Compound **3** (**1 g**) was isomerized as per general procedure by using $MgI_2.Et_2O$. After purification one major isolable product **6** was obtained. The

product was characterised as furostenol pseudo-sarsasapogenin diacetate **6** (700 mg); Mass: m/z 500 (M^+), 440, 380, 364; IR: 1735 (two acetate groups), 1400, 1250 cm^{-1}; ^1H NMR: δ 1.1 (bs, 3H, C-18 methyl), 0.9 (bs, 6H, C-19 & C-25 methyl), 1.8 (bs, 3H, methyl on double bond), 2.0 (bs, 6H, acetate protons), 3.8 (d, 2H, *J*=7.5 Hz, C-26 methylene protons).

Similarly, compound **3** (1 g) furnished the product **6** (650 mg) where TMSCl was used in place of magnesium iodide ethereate in the above reaction.

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Indian Journal of Chemistry
Vol. 37B, April 1998, pp.411 - 414

Note

Electrogenerated superoxide induced oxidation of thiols and disulfide interchange : A functional group approach to biomimetic study

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Received 29 April 1997; accepted (revised) 21 October 1997

Thiols are readily oxidized to disulfides using electrolytically generated superoxide ion in dimethylformamide. Unsymmetrical disulfides are obtained as a result of interchange between two different symmetrical disulfides under the same set of reaction conditions.

Detection and quantification of superoxide radical anion, $O_2^{-\cdot}$, in living bodies have become quite important in connection with the recent explosive development of free radical biochemistry and medical sciences[1]. The formation of *in vivo* $O_2^{-\cdot}$ is a result of one electron reduction of molecular oxygen and its presence can be tested by the use of superoxide dismutase (SOD)[2] - an enzyme which plays a protective role within the cell by reducing steady - state levels of $O_2^{-\cdot}$. Superoxide mediates important physiological processes and simultaneously may induce the cellular damage leading to various pathologies[3,4]. While the heated studies regarding superoxide's modes of biological action have provoked a vivid interest in this species, there is yet a dearth of information regarding its *in vivo* role based on chemical findings[5]. As a result, the studies are directed to explore the reactions of *in situ* generated $O_2^{-\cdot}$ with vital organic substrates which may ultimately serve as a model for those encountered in more complex living systems.

Superoxide is a potent oxidizing agent and as such could affect cellular function by oxidizing low molecular thiols and proteins containing the sulfhydryl groups[6-10]. Glutathione, cysteine and other thiols are also oxidisable by the $O_2^{-\cdot}$ produced by stimulated leucocytes[11]. The $O_2^{-\cdot}$ produced by xanthine oxidase further reacts with the thiol-containing nucleic acid base 6-mercaptopurine[12]. In view of the above, the chemistry along the way appears to be very significant. Scanning of literature reveals that there exist some reports dealing with the reaction of organic sulphur compounds with potassium superoxide and 18-crown-6 ether[13-15]. However, to the best of our knowledge, the reactivity of thiols with electrolytically generated $O_2^{-\cdot}$ is not known and has been undertaken in view of the following reasons : (i) electrolytic reduction of molecular oxygen (O_2) in aprotic media provides a pure and stable solution of *in situ* generated $O_2^{-\cdot}$ in a controlled fashion. Since only part of O_2 supplied to the cathode compartment is reduced, the intermediates have the opportunity directly upon their generation to react with excess dissolved O_2. This method is well suited for bio-mimetic studies. (ii) The commercially available KO_2 is only 96% pure and may yield solutions that are contaminated with varying amount of K_2O_2 and KOH. The slow decomposition of crown ethers in these solutions has also been noted. These facts thereby delimit the interpretation of results.

In view of the above and as a part of our continued interest[16-19] on superoxide chemistry, we wish to report our findings on the reactivity pattern of *in situ* electrogenerated $O_2^{-\cdot}$ with some simple thiols **1** as model from the functional group perspective (Scheme I).

$$O_2 \xrightarrow[DMF,\ (n\text{-}Bu)_4NClO_4]{Hg\ Cathode,\ -0.9\ V\ vs\ SCE} O_2^{-\cdot}$$

$$R\text{-}SH \xrightarrow{O_2^{-\cdot}\ /\ O_2} R\text{-}S\text{-}S\text{-}R$$

1 **2**

Scheme I

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In the course of reaction, $O_2^{-\cdot}$ is produced by univalent reduction of molecular oxygen[20,21] at a constant potential of -0.9 V vs SCE at mercury cathode in DMF and is subsequently allowed to react with the substrate **1**. As an outcome (*cf.* Table **I**), ethanethiol **1a**, butanethiol **1b**, benzenethiol **1c**, 4-methylbenzenethiol **1d**, 4-methoxybenzenethiol **1e**, α-toluenethiol **1f**, 4-chlorobenzenethiol **1g**, 2-aminothiophenol **1h** and cyclohexyl mercaptan **1i** are oxidatively transformed into their corresponding disulfides as the only isolable product. Whereas, the dithiols such as, ethane-1,2-dithiol **1j**, propane-1,3-dithiol **1k** and toluene-3,4-dithiol **1l** formed cyclic sulfur compounds viz., 1,2,5,6-tetrathiocan **2j**, 1,2,6,7-tetrathiecan **2k** and dimethyldibenzo- (1,2,5,6)-tetrathiocin **2l** respectively. Each reaction was monitored by TLC and showed in almost all cases the total disappearance of the starting materials. All the products **2a-l** exhibited physical and spectral data (IR & ^{1}H-NMR) consistent with their structures.

Considering the greater acidity of S-H bond, the mechanism of these reactions probably involves initial deprotonation followed by oxidation of the resulting anion and radical coupling successively (*cf.* Scheme II).

$$R\text{-}SH + O_2^{-\cdot} \rightarrow R\text{-}S^{\cdot} + HO_2^{\cdot}$$

$$HO_2^{\cdot} + O_2^{-\cdot} \rightarrow HO_2^{-} + O_2$$

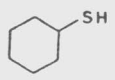
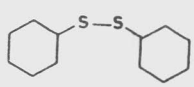
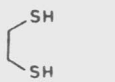
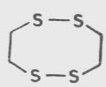
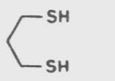
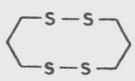
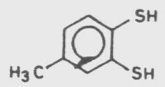
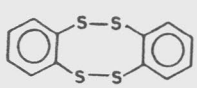
$$R\text{-}S^{\cdot} + O_2 \rightarrow R\text{-}S^{+} + O_2^{-\cdot}$$

$$2R\text{-}S^{\cdot} \rightarrow R\text{-}S\text{-}S\text{-}R$$

Scheme II

Further, suproxide is a potent reducing agent and as such could affect cellular function by reducing disulfides in important proteins, such as, ionic channels and pumps. In support of this concept, the reduction of disulfide bonds by $O_2^{-\cdot}$ has been shown in an *in vivo* model[22]. As the disulfide linkage plays a key role in the living systems, there exist some reports regarding the interchange reactions of disulfides[23-25]. Prompted

Table I—Reaction of electrogenerated $O_2^{-\cdot}$ with thiols **1a-l**

1, 2	Substrate 1	Product 2	Yield* (%)	m.p./(b.p.) °C Found	m.p./(b.p.) °C Reported[14,15,26,27]
a	CH_3CH_2-SH	$(CH_3CH_2\text{-}S)_2$	61	(44-6/10mm)	(46/11 mm)
b	$CH_3(CH_2)_3$-SH	$(CH_3(CH_2)_3\text{-}S)_2$	54	(104-6/10mm)	(102-4/9 mm)
c	C_6H_5-SH	$(C_6H_5\text{-}S)_2$	82	59-61	61
d	4-$CH_3C_6H_4$-SH	$(4\text{-}CH_3C_6H_4\text{-}S)_2$	77	45-47	48
e	4-$CH_3OC_6H_4$-SH	$(4\text{-}CH_3OC_6H_4\text{-}S)_2$	80	43-44	44-45
f	$C_6H_5CH_2$-SH	$(C_6H_5CH_2\text{-}S)_2$	74	70-71	69-70
g	4-Cl C_6H_4-SH	$(4\text{-Cl } C_6H_4\text{-}S)_2$	72	70	70-71
h	2-$NH_2C_6H_4$-SH	$(2\text{-}NH_2C_6H_4\text{-}S)_2$	68	91-92	93
i			63	(180-82/10mm)	(195/20 mm)
j			58	147-49	151-52
k			52	69-70	71
l			45	189-91	192-95

* Isolated mass yield based on **1** as weight %.